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FRANK, CUBE AND TETRAHEDRON VCG AND CONVENTIONAL ECG IN VENTRICULAR OVERLOAD

CORRELATIONS BETWEEN SELECTED QRS AMPLITUDE MEASUREMENTS AND HEMODYNAMIC PARAMETERS

BY

HEIKKI RIEKKINEN

HELSINKI, FINLAND 1971

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INTRODUCTION AND PURPOSE OF THE STUDY

Heart catheterization and X ray kinemato graphic procedures are generally used in clinical applications, and they are considered reasonably accurate techniques for determ nation of the degree of ventricular pressure and volume overload However a certain risk is involved w th these p ocedu es and non traumatic methods would be em nently prefer able if only they could be pro en ccurat enough fo clinical purposes.

The most commonly applied non tra matic methods in clin cal cardiology include conventio-nal electrocardiography (ECG) and ector cardiography (VCG) which both ha e been claimed to be valuable for prediction of the degree of entricular loading But most of the studies performed concern groups of subjects with pressur overload only of th right or the left entricl No integrated assa exist of subjects with different types of entricular o erload, and no report ha e been published on the prediction of the relativ overload ratio of the entricles. It also ppears that bi entricular overload ha been inadequately investigated

Theoretically corrected lead stems ar superior to the uncorrected lead systems, but no definite es lts can be cited to prove better correlation on hemodynamic measur ments of mea urements and criteria derived from corrected lead s tems compared to those from the uncorrected lead systems. Relatively high correlation ha been reported in normal subjects fo equivalent QRS amplitude mea ure ments from uncorrected and corrected leads but no imila studies of clinically abnormal subjects with hemod namic overload of the entricles have been m de

The purpose of the present sudy can be outlined a follows

1 To investigate correlations of selected QRS amplitude measurements from th C be and Tetrahedron VCG lead to the corres ponding measu ements from the Frank VCG leads in group of clinically abnormal subjects with w de spectrum of d fferent types of hemodynamic overload of the en tricles. The items selected for these orrelation include those primary nd secondary amplitude parameters which ha been commonl used as criteria for elative right or left entricular predom nance Certain equivalent data f om the con entional 12 lead ECG are also included in the correlation analysis.

2 To in estigate the extent to which it is pos ible to p edict right and left entricular overload and the elativ overload ratio of the entricles, in terms of selected hemod nam parameters, f om selected, commonly used parameters and certain new ones derived f om the Frank, Cube and Tet hedron VCG lead and conventional ECG leads, in the following series

a The compos te group of subject with wid spectrum of d fferent t pes of vent cula overload selected from the members of group b c and d specified below n whom both right and left entricular hemod namic me surements were lable

b Subject w th a heart disease resulting in right entricular overload,

c. Subjects w th heart disease resulting n left entricula overload, and

d Subjects with a heart disease resulting in bi entricula overload.

REVIEW OF THE LITERATURE

Lead systems

The conventional 12 lead system has been accepted as standard in clinical electrocardiography whereas in vectorcardiography there is no commonly accepted standard.

The first vectorcardiographic lead systems were introduced by Schellong (Schellong et al 1937), Wilson (Wilson and Johnston 1938) and Kimura (Kimura 1939). Wilson used the Tetrahedron system and Schellong et al. and Kimura Cube systems.

In all Cube systems distant electrode locations are used in order to avoid or minimize the so-called proximity effect which could invalidate the adequacy of dipole approximation. Cube systems were also assumed to be more orthogonal than the Tetrahedron system. Most of the early VCG lead systems used a cube as *their reference frame* (Duchosal and Sulzer 1949, Grishman and Scherlis 1952 and Milnor et al. 1953).

It was found quite early that electrical distortion occurred in the lead systems in use (Burger and van Milaan 1946, 1947 and 1948, Schmitt 1953, Frank 1954, Frank and Kay 1954 and Schmitt and Simonson 1955). It was also noted that most lead systems in use at that time were susceptible to variations in anatomic location of the equivalent dipole from one *subject to another (Frank 1956).*

On the basis of the results gained by experiments with torso models new "corrected" lead systems were developed to reduce these distortions. Two of the first of these systems were the SVEC III lead system (Schmitt and Simonson 1955) and the Frank lead system (Frank 1956). An attempt has been made in them to correct the errors arising from individual variabilities in torso shape and dipole location by means of more numerous electrodes and resistance network to combine the potential differences from the electrodes in given proportions. Basic assumptions underlying these systems are that the ventricular depolarization can be adequately represented at any time by an equivalent dipole which is variable in strength and orientation but fixed at a single location for each individual, and that the medium in which the *heart currents are* produced *homogeneous.*

Some of the other corrected lead systems are the Helm lead system (Helm 1957) which uses large sponge electrodes, *the axial McFee* Parungao lead system (McFee and Parungao 1961) and the Barber Fischmann lead system (Barber and Fischmann 1961) which uses grid electrodes, i.e. a field of multiple electrodes on the chest. These systems, too, are mostly on results from experiments with torso models.

Many other corrected and uncorrected lead systems exist in addition to those mentioned.

Wilson's Tetrahedron system (Wilson and Johnston 1938) utilizes Einthoven's standard leads. *The sagittal and horizontal components are* obtained with the aid of one additional electrode located 2 cm to the left of the spinous process of the seventh thoracic vertebra. The Tetrahedron *lead system* is one of the oldest systems still in use. Its greatest advantage is that the electrode locations are easy to determine anatomically. Its principal disadvantage is relatively poor electrical orthogonality (Frank 1956). Burch and De Pasquale have accumulated a very extensive material using the Tetrahedron system.

Grishman lead system (Grishman and Scherlis 1952) employs a cube as reference frame. The electrodes of three bipolar leads are applied on the chest to form three adjoining edges of a cube in order to produce three orthogonal scalar components of the VCG. Grishman's

Cube system is perhaps the uncorrected lead system in most common use to-day It is anatomically orthogonal b t electrically con siderably distorted (Frank 1956)

The F ank lead system (Frank 1956) is a corrected VCG lead system which makes use of 7 electrode locations. Five electrodes are placed on the same horizontal level on the chest, one on the neck and one on the left leg

The Frank lead system is perhaps the most popular corrected lead system in use to-day It is theoreticall, superio to uncorrected syst ems because the orthogonal ty equirement is reasonably well satisfied at least in a homo geneous torso. One of its disadvantages is that the electrode placement is rather critical The electrode sites should be well prepa ed when th *system is used so that distortions produced by* high skin-electrode interface esistance might be avoided. It should also be noted that th same problem may arise in many other ystems, including conventional ECG leads, unless proper precaution is taken in the design of the recording system.

Comparison of lead systems

Marked differences n the shape of the vector loops determined by different lead systems have been extensively reported (Burger et al 1952, Burger et al. 1956 Frank and Seiden 1956 Dower and Osborne 1958 Burger et l 1959 Burger et al. 1961 Burger et al 1962 Hatton 1965 a and b and Karobath and Wenger 1967). This reflects significant phase and ampl tude d fferences and, correspondingly QRS ampl tude measurements from the scalar components and ecto loop of different leads have been eported to ary great deal (Schaffer et al. 1953 Simonson et al. 1955 Ab ldskov and Pence 1956 Simonso et al 1957 Simonson et al 1959 Hugenholtz nd Liebman 1962 Yokoama 1963 Beswick nd Jordan 1964 Gunther and Graf 1965 Lan Scheng et al 1965 Amger 1967 Hänninen 1967 Pawlov 1967 Amger 1968 nd Borun 1968) QRS a nplit ides determ ned f o corrected leads has been eported to deviate less from the theoretically expected ampl tudes than those derived from uncorrected lead (B ody 1964 F schmann 1965 Nonogawa 1965 Brody and Arzbaecher 1966 Nonogawa 1966 Yamada and Okajima 1967 and Helm and Chou 1969)

Relatively high correlations have been found between corresponding QRS amplitude measure ments from different corrected leads (Beswick and Jordan 1964 Horan et al 1965 and Borun 1968) but very few authors, with notable exception of the invest gations by P pberger and Lilienfield (1958) and Borun et al (1969) have studied the correlat on between eq ivalent QRS ampl tude measurement from corrected and uncorrected leads. Borun et al. (1969) reported relati ely h gh correlations n normal ch ld en between Ry (= 0.84) Sy (r = 0 76) and Rz (= 0 77) but relatively low ones between Rx (= 0.37) Sx (= 0 58) and Sz (r = 0.52) mea *sured from the Frank nd Cube leads*

Vector loops ha e been repo ted to l superior to the conventional 12 lead ECG in the diagnosis of ventricular hypertrophy by som a thors (Belyaeva and Namazova 1965 Lee et al. 1965 Kovács Hopff and Wys 1966 and Brown 1968) This is, however opposed by the find ngs of some other investigators (S monson et al. 1966 a and Mizuno and Yasu 1967) Okamoto (1967) reported the SVEC III leads to be superior to the Cube leads i the diagnosis of right ventricula hypert ophy based on the shape of the ector loop but Morse et al (1966) d d not find any sign ficant difference between the diagnostic capacity of the Frank and Duchosal Sulzer lead system in the diagnosis of left entricular hypertrophy

On the basis of pattern classification, closer relation to changes of some particula hemo dynamic parameters ha been reported fo measurement from VCG leads than f om the on entional ECG leads (Elek et al. 1954 Donoso et al 1955 Baedeker et al 1963 and Cueto et al 1966) and for those f om corrected leads, comp red to uncorrected lead (Baedeker et al 1964 and Okamoto 1967) S lverblatt et al (1957) however found no consistent relationship between right entricula systolic peak pres ure and patterns of the con entional ECG or Cube VCG n subjects with atrial septal defect.

In quantitative studies, better correlation on some particular hemodynamic parameters has been reported fo QRS mplitude measurement from VCG leads than from the conventional

ECG leads (Gamboa et al. 1965 Gamboa et al. 1966 and Vine et al. 1969) and for those from corrected leads, compared to uncorrected leads (Gamboa et al. 1965 and Gamboa et al 1966) Powell et al. 1969), however found no distinct difference between the correlations of selected QRS amplitude measurements from the Helm VCG leads and from the conventional ECG leads on left ventricular systolic peak pressure in subjects with aortic stenosis or coarctation. Details concerning these studies will be cited in the next section

ECG and VCG in ventricular overload

In subjects with right ventricular pressure overload a relation has been found between selected QRS amplitude measurements and the severity of overload on the basis of pattern classification (Johnson et al 1950 Elek et al 1954 Donoso et al. 1955 Silverblatt et al 1957 Wasserburger and Brown 1958 Kahn et al. 1959 Yahini et al 1960 Luna and Crow 1961 Baedeker et al. 1963 Scherlis et al 1963 Strang et al. 1963 Benchimol and Lucena 1965 and Doring and Trenckmann 1965) Also quantitative correlations between selected QRS amplitude measurements and particular hemodynamic parameters have been reported (Bass-thwaighte et al. 1963 Hugenholtz and Gamboa 1964 Hava h 1966 Gamboa et al. 1966 W tham et al 1965 and Holt et al 1969 In some of these studies the corre lations were elatively high Hugenholtz and Gamboa 1964) studied 40 children and young adults w th pure congenital pulmonary stenosis, using the Frank lead system. They found a oefficient of 0 87 for the correlation between ight entricular s stolic peak p essu e and maximum rightward spatial vector Gamboa et al 1966 found a coefficient of 0 85 for he orrel tion between right entricular syst olic pe k pressu nd maximum rightward spat ial ect r mea ed f om the Frank lead in 50 pa ients wit onge ital alvular pulmonary [illegible] ears. The orresponding elation oefficien f r Cube lead me u eme w 5(f n l m S1 RV1 SV6 measu ed f m th con entio nal ECG le d it was 0 60 Witham et al. (1968) studied 21 patients with pulmonary stenosis between 1 and 55 years of age using the Helm lead system. They found a coefficient of 0 90 for the corre lation between right ventricular systolic peak pressure and the spatial sum of the term inal (S) vectors.

Gordon and Goldberg (1951) found no significant correlation between right ventricular systolic peak pressure and the amplitudes of the R, S, and R wa es and the R/S ratios in V1 V5 and V6 in subjects with "right heart strain pattern" in the conventional ECG

Those studies are far less in number which concern correlations between ECG or VCG measurements and hemodynamic parameters in subjects with right ventricular olume overload compared to those relating to pressure over load. On the basis of pattern classification a relation between selected QRS amplitude mea surements and the severity of the right ventri cular overload has been observed (Walker et al. 1956, Toscano-Barboza et al. 1958 de Ol veira and Zimmermann 1958 Lee and Scherlis 1962 Chabot et al. 1965 Brown et al. 1968 Gault et al. 1968 and Siltanen 1969) Hayashi et al. (1966) using the Frank lead system, found coefficients of 0.562 and 0 517 for the corre lations between right ventricula systolic peak pressure and maximum rightward and max imum anterior deviation of the QRS loop respectively in 28 subjects with atrial septal defect. Murata et al (1968) found a coefficient of 0 75 for the correlation between right entricular systolic peak pressure and the maximum rightward/leftward vector rat o mea sured from the Frank lead VCG in 31 patients with atrial septal defect.

In left entricular overload comparatively high correlations between selected QRS ampl t ude measurements and left entricular systol c peak pressure have been reported by some authors. Hugenholtz et al. (1962) reported sign ficant correlation (r = 0.77) between left ventricular peak systolic pressure and the magni tude of the maximum QRS ector in the horizontal plane n 40 children and young adults with congenital aortic stenosis, and in 45 ontrols (by Cube lead system) Hugenholtz and Gamboa (1964) studied 50 children and oung adults with aortic stenosis employing the Frank lead system. They found a coefficient

of 0 85 for the correlation between left entricular systolic peak pressure and maximum spatial vector Gamboa et al (1965) found a relatively high correlation (r = 0 88) between left ventricular systolic peak pressure and the maximum spatial vector measured from the Frank lead VCG in 30 ch ldren and young adults with congen tal aortic stenosi The correlation on the corresponding Cube lead measurements and on the sum SV2+RV5 meas ured from the conventional ECG leads were lower (r = 0 72 and 0 48 respectively) Pottell et l. (1969) studied ? patients with aorti sten si o ort c co ctat on between 6 and 70 years, using the conventional ECG and the Helm lead VCG They found about as good correlaton between the sum SV1+R,SaVF from the conventional ECG leads and left ventricular systolic peak p essure (r = 0 72) as between the spatial R voltage from the Helm lead VCG and left ventricular systolic peak pressure (r = 0 70) Reeve et al. (1966) however found no sign ficant corr lation between the maxium spatial vector measured from the Frank lead VCG and left ventricula systolic peak pressure in 8 patients with congenital ortic stenosis ged from 5 to 26 years.

In subjects with a cond tion leading to biventricular overload a relation ha been found on the basis of pattern classificat on between the severity of right or left entricular over load and selected QRS ampl tude measurements (Hubbard and Angle 1957 Char et al. 1959 Toscano-Barboza and Dushane 1959 Beregovich et al 1960 Bu ch and DePasquale 1960 Scott 1961 Vince and Keith 1961 Arnaud 1965 Khoury et al. 1966 and B ch and DePasquale 1967) There are however very few studies on the correlations between selected QRS amplitude measurements nd specific hemo dynamic parameters n biventricula overload Papadopoulos et al (1965) found a poor corre lation between the height of the R wa e n lead V1 and right entricular sy tolic peak pressure in 50 patients, aged f om x months to 45 years, with isolated ventricular septal defect. To th p esent utho best knowledge no significant correlations ha been reported in adults at least

ECG lead (Gamboa et al 1965 Gamboa et al 1966 and Vine et al 1969) and for those from corrected lead compared to uncorrected lead (Gamboa et al 1965 and Gamboa et al 1966). Pe sell et al 1967) however found no distinct difference between the correlations of selected QRS amplitude measurements from the Helm VCG lead and from the conventional ECG lead on left ventricular systolic peak pressure in subjects with aortic stenosis or coarctation. Detail concerning these studies will be cited in the next section.

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Gordon and Goldberg (1951) found no significant correlation between right ventricular systolic peak pressure and the amplitudes of the R S and R waves and the R/S ratios in V1 V5 and V6 in subjects with right heart strain pattern in the conventional ECG.

Those studies are far less in number which concern correlations between ECG or VCG measurements and hemodynamic parameters in subjects with right ventricular volume overload compared to those relating to pressure overload. On the basis of pattern classification a relation between selected QRS amplitude measurements and the severity of the right ventricular overload has been observed (Walker et al 1956 Toscano Barbosa et al 1958 de Oliveira and Zimmermann 1958 Lee and Scherlis 196[illegible] Chabot et al 1965 Brown et al 1965 Gault et al 1968 and Siltanen 1969). Hayashi et al (1966), using the Frank lead system, found coefficients of 0.562 and 0.517 for the correlations between right ventricular systolic peak pressure and maximum rightward and maximum anterior deviation of the QRS loop respectively in 78 subjects with atrial septal defect. Murata et al (1968) found a coefficient of 0.75 for the correlation between right ventricular systolic peak pressure and the maximum rightward/leftward vector ratio measured from the Frank lead VCG in 51 patients with atrial septal defect.

In left ventricular overload comparatively high correlations between selected QRS amplitude measurements and left ventricular systolic peak pressure have been reported by some authors. Hugenholtz et al (196[illegible]) reported significant correlation (r = 0.77) between left ventricular peak systolic pressure and the magnitude of the maximum QRS vector in the horizontal plane in 40 children and young adults with congenital aortic stenosis, and in 45 controls (by Cube lead system). Hugenholtz and Gamboa (1961) studied 50 children and young adults with aortic stenosis, employing the Frank lead system. They found a coefficient

Table 2. Diagnoses and age distribution of 41 subjects w th a heart disease leading to right ventricular overload.

Diagnosis	Age group (years)					
	16—20	21—30	31—40	41—50	1—53	Total
Atrial septal defect of secundum type	4	8	8	4	—	4
Valvular pulmonary stenosis	3	5		—	—	10
Mitral stenosis	—	—	—	2	1	3
Infundibular pulmonary stenosis	—	—	—	1	—	1
Valvular and infundibular pulmonary stenosis	1	—	—	—	—	1
Cor pulmonale	—	—	—	—	1	1
Essential pulmonary hyper tension	—	—	1	—	—	1
Total	8	13	11	7	2	41

Right ventricular overload

The diagnoses and the age d stribution of the 41 subjects with a heart disease leading to right ventricular overload are shown n Table 2 In 17 subjects a heart disease leadi g to right entricular pressur o erload w diagnosed and in 24 subjects a heart disease leading to right entricular ol me overload They were classified as ha ng right entricula pressur and olume o erload espectivel

Table 3. Diagnoses and ge distribution of 28 subjects with a heart d sease leading to left ventricular overload.

Diagnosis	Age group (years)					
	16—20	21—30	31—40	41—50	51—59	Total
Aortic st nosi	1	2	2	5	2	12
Aortic insufficiency	1	1	3	4	1	10
Aortic tenosis and nsufficiency	—	1	—	1	1	3
Aortic coarctation	—	2	1	—	—	3
Total	2	6	6	10	4	28

Left ventricular overload

The diagnoses and the age di tribution of the 8 sul ject with a he rt disease lead ng to left ventricular verl d are shown in Table 3. They were cla ified as having left ventricular verload.

Biventricular overload

The diagnoses and the age distribution of the 23 sul ject wit a heart disease leading to biventricular overl ad are shown in Tabl 4. They were classified a having biventricular overload.

Table 4. Diagnoses and age di tribution of 23 subject with a heart disease leading to biventricular overload.

Diagnosis	Age group (years)					
	17—20	21—30	31—40	41—50	51—5	Total
Ventricular septal defect	1	4	1	—	—	6
Patent ductus arteriosus	—	1	1		—	4
Eisenmenger complex	1	2	—	1	—	4
Aortic and mitral valve disease	—	—	—	4	1	5
Aortic or mitral valve disease in combination with pulmonary stenosis or atrial septal defect	3	1	—	—	—	4
Total	5	8		7	1	23

Other subjects

No heart d sease wa found in two men nd one woman aged 18, 31 and 16 years respectively. In the correlat on analysis these ubject were included n the omposite group of subjects in whom the right well a left ent ula tems on t me in lex and the La n l ad t ould be determ ned. I dd n, 4 sul ject w th r ou he rt diseases and various degrees of ventricular loading were included as subjects in the study on correlation of selected QRS amplitude measurements from the Cube and Tetrahedron VCG leads and from the conventional ECG lead on the corresponding measu ements from the Frank VCG leads. Altogether the group f ul ject selected f this ECG VCG rre lation analy is consisted of 137 patient with a wide pectrum of different types of hemo dynamic load of the ventricles.

METHODS

Vectorcardiography and electrocardiography

Equipment and procedur s

Vectorcardiographic and electrocardiograph c recordings were made on the day after or before catheterization.

The Frank, Cube and Tetrahedron lead VCG phase plane projections, which are here called loops, were recorded in th s order in supine subjects using the Sanborn Vector Ampl fier Model 185 and the Sanborn Visoscope Model 169 together with a Frank lead resistor network (F ank Box, Sanborn) The value of the resistor R in the network was 100 000 ohms The time interval between the direction identif cation gnal n the vecto loops wa 0 0025 sec.

The frontal, left sagittal and horizontal plane QRS loops were photographed from t least two beats on the oscilloscope using a Hewlett Packard Oscilloscope Camera Model 196 A. In connection of every ecord cal bration signal of one millivolt was applied and verified The subjects were instructed to breathe quietly during the recording

The chest electrodes of th Frank lead were placed at the horizontal level of the fourth intercostal space at the sternal margin This level was marked with the aid of plumb bob around the chest of the subjects in upright position The position of electrode C was corrected n sup ne subjects if a mark d deviation in the location f om that in upright pos tion was noticed. The electrodes of the Cube leads were placed s ecommended by Grishmann and Scherlis (1952) Th inter electrod dista es w k pt l nel equal as was practicabl Th electrodes of the Tetrahedron leads were placed as ecommended by W lson and Johnston (1938)

Plate electrodes, 3 x 5 cm in d amete (El ma Schönander) were used Electrode paste (Electrodyne) was applied on th electrodes and the k n under the electrodes was rubbed until it was clearly flushed, or for t least 30 sec.

The conventional 1 lead ECG was recorded immediately after recording the VCG using a direct reco ding Mingograf 81 (Elema Schon ander) w th input impedance of 100 megaohms A one millivolt cal bration signal was reco ded on every channel

All VCG reco d ngs and the majority of th ECG recordings were made by the uthor The rest of the ECG record ngs were made by well trained n rse

ECG and VCG measurements

A set of primary QRS ampl tude measure ments, and certain sums and quotient derived from the primary measurements, were selected fo correlation analysis

The following param ters wer used as ind ces of right entricular preponderance

1 Sx, defined the absol te alue of the minimum of the X component during th second half of the QRS nterval

2 FRMV (frontal plane rightward maxim m ecto) def ned as the absolute alue of th maximum frontal plane ecto on the right side of the Y axis duri g the second half of th QRS interval

3 HRMV (horizo tal plane rightwa d maxim m vector) defined as the bsol te value of the maximum horizontal plan vector on the right side of the Z axi during the second half of the QRS interval

4 RMSV (rightward maximum spatial vect o) defined as the absol te value of th n tant neou QRS ecto t th t se po t

Left ventricular overload

The diagnoses and the age distribution of the 23 subjects with a heart disease leading to left ventricular overload are shown in Table 3. They were classified as having left ventricular overload.

Biventricular overload

The diagnoses and the age distribution of the 23 subjects with a heart disease leading to biventricular overload are shown in Table 4. They were classified as having biventricular overload.

Table 4. Diagnoses and age distribution of 23 subjects with a heart disease leading to biventricular overload.

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Eisenmenger complex	1		—	1	—	4
Aortic and mitral valve disease	—	—	—	4	1	5
Aortic or mitral valve disease in combination with pulmonary stenosis or atrial septal defect	3	1	—	—	—	4
Total	5	8	2	7	1	23

Other subjects

No heart disease was found in two men and one woman aged 18, 31 and 16 years, respectively. In the correlation analysis these subjects were included in the composite group of subjects in whom the right as well as left ventricular tension time index and the ventricular overload ratio could be determined.

In addition, 12 subjects with various heart diseases and various degrees of ventricular loading were included as subjects in the study on correlations of selected QRS amplitude measurements from the Cube and Tetrahedron VCG leads and from the conventional ECG leads on the corresponding measurements from the Frank VCG leads. Altogether the group of subjects selected for the ECG-VCG correlation analysis consisted of 137 patients with a wide spectrum of different types of hemodynamic load of the ventricles.

METHODS

Vectorcardiography and electrocardiography

Equipment and procedures

Vectorcardiographic and electrocardiographic recordings were made on the day after o before catheterization.

The Frank, Cube and Tetrahedron lead VCG phase plane projections, which ar here called loops, were recorded in this order in supine subjects using the Sanborn Vecto Amplifier Model 185 and the Sanborn Visoscope Model 169 together with a Frank lead resistor network (Frank Box, Sanborn) The value of the resistor R in the network was 100 000 ohms The time nterval between the direction identification signals in the ecto loops w 0 0025 sec

The frontal left sagittal and horizontal plane QRS loops were photographed from at least two beats on the oscilloscope u ing a Hewlett Packard Oscilloscope Camera Model 196 A In onnection of every record a cali bration signal of one millivolt was applied and verified. The subjects were instructed to breath quietly during the recording

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Plate lectrodes, 3 x 5 cm n diameter (Ele ma Schönander) were used Electrode past (Electrodyne) was applied on th elect odes and the skin under the electrodes was rubbed until t was clearly flushed, o fo at least 30 sec

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All VCG reco dings and the majority of the ECG reco d ngs were made by the uthor The rest of the ECG record ngs were made by a well trained nurse

ECG and VCG measurements

A set of primary QRS mplitude measure ments, and certain sums and quotients derived from the primary measurements, were selected for correl tion analysis.

The following parameters were used as indices of right ventricular preponderance

1 S defined as the absol t alue of the m nimum of the X component duri g th second half of the QRS nterval

2 FRMV (frontal plane rightwa d maximum ecto) def ned as the absolute alue of the maximum frontal plane ector on the right side of the Y axis during the second half of the QRS interval

3 HRMV (horizontal plane rightward maximum ector) defined as the absolute alue of the maximum horizontal plan ector on the right side of the Z axis during the second half of the QRS nterval

4 RMSV (rightward maximum spatial vect o) defined as the absolute value of the instantaneous QRS ctor at tl t out

f the minimum value (peak of S) of the X component during the second half of the QRS interval. This vector is selected as an approximation for the maximum spatial vector located on the right side of the YZ plane

5 FRMV+HRMV

6 SI defined as the amplitude of the S deflection in the conventional lead I

7 R,R'V1 defined as either RV1 or R'V1 whichever is larger and

8 SI+R'V1+SV6

The following parameters were used as indices of left ventricular preponderance

9 FLMV (frontal plane leftward maximum vector) defined as the absolute value of the maximum frontal plane vector on the left side of the Y axis

10 HLMV (horizontal plane leftward maximum vector) defined as the absolute value of the maximum horizontal plane vector on the left side of the Z axis

11 LMSV (leftward maximum spatial vector) defined as the absolute value of the instantaneous QRS vector at the time point of the maximum value (peak of R) of the X component. This vector is selected as an approximation for the maximum spatial vector located on the left side of the YZ plane

12 FLMV+HLMV

13 RI+SV1

14 RI+R,SaVF with R,SaVF defined as RaVF or SaVF whichever is larger

15 SV1+RV5,6 with RV5,6 defined as RV5 or RV6 whichever is larger

16 SV1+R,SaVF

17 RI+SV1+RV6 and

18 RI+R,SaVF+SV1

The following parameters were used as indices for the ventricular preponderance ratio (VPR)

19 Sx/(Sx+Rx)

20 Rz/(Rz+Sz)

21 FRMV/(FRMV+FLMV)

22 HRMV/(HRMV+HLMV)

23 RMSV/(RMSV+LMSV)

24 (FRMV+HRMV)/((FRMV+HRMV)+(FLMV+HLMV))

25 SI/(SI+RI)

26 R,R'V1/(R,R'V1+SV1) and

27 (SI+R'V1+SV6)/((SI+R'V1+SV6)+(RI+SV1+RV6))

The following parameters were moreover used in amplitude correlations

28 Rx defined as the maximum value of the X component

29 Ry defined as the maximum value of the Y component

30 Sy defined as the absolute value of the minimum of the Y component during the second half of the QRS interval

31 Rz defined as the maximum value of the Z component during the first half of the QRS interval

32 Sz defined as the absolute value of the minimum of the Z component during the second half of the QRS interval

33 Sy/(Sy+Ry)

34 RV5

35 SV5

36 RaVF

37 SaVF

38 RV

39 SV

40 SV5/(SV5+RV5)

41 SaVF/(SaVF+RaVF) and

42 RV.../(RV...+SV...)

The polarity of the leads of all three VCG lead systems was chosen in conformity with the polarity of the conventional leads I, aVF and V1 so that the X, Y and Z leads were positive in the right to left, down to up and posterior to anterior directions, respectively.

Hemodynamic methods and measurements

Equipment and procedures

The heart catheterizations were made according to the routine procedures followed in our laboratory. The level 10 cm over the table surface was taken as zero reference level for the pressure recording in supine subjects. Left heart catheterizations were made by transbrachial technique in most subjects. All subjects were in postabsorptive fasting state and most subjects were premedicated with 200 mg Antistin® about one hour previously. Details of the catheterization technique used in our laboratory have been presented by Siltanen (1969).

Hemodynamic measurements

The right and left ventricular tension times per beat, defined as the area under the systolic portion of the ventricular pressure curve, were measured by planimetry from 3 representative beats in most subjects. In a few of the records only two cardiac cycles were used for this measurement. The right and left ventricular tension time index (TTIRV and TTILV) were calculated as the product of heart rate and tension time per beat. In some of the subjects no ventricular pressure record was available and TTILV was calculated from the tension time per beat measured from the aortic or brachial arterial pressure curve.

The ventricular overload ratio (VOR) was defined as the ratio TTIRV/(TTIRV+TTILV) where TTIRV and TTILV are the right and left ventricular tension time index, respectively.

Statistical analysis

In the whole group of 137 subjects the correlations of selected QRS amplitude measurements from the Cube and Tetrahedron lead VCG on the corresponding measurements from the Frank lead VCG were determined. The correlations of certain corresponding items derived from the conventional 12 lead ECG on the Frank lead measurements were also determined. The measurements selected are listed in Tables 5 and 6 in the Results section.

In the group composed of all 81 subjects in whom both TTIRV and TTILV were determined, the correlations on VOR of selected QRS amplitude measurements from the Frank, Cube and Tetrahedron lead VCG's and from the conventional 12 lead ECG were determined. The measurements selected are listed in Table 7 in the Results section.

In the 41 subjects with a heart disease leading to right ventricular pressure or volume overload the correlations of selected QRS amplitude measurements from the Frank, Cube and Tetrahedron lead VCG and from the conventional 12 lead ECG on TTIRV and on VOR were determined. The same correlations were also separately determined for the 17 subjects with a heart disease leading to right ventricular pressure overload and for those 24 who had heart disease leading to right ventricular volume overload. The measurements selected are listed in Table 8 in the Results section.

In the 28 subjects with heart disease leading to left ventricular overload the correlations of selected QRS amplitude measurements from the Frank, Cube and Tetrahedron lead VCG and from the conventional 12 lead ECG on TTILV were determined. The measurements selected are listed in Table 11 in the Results section.

In the 23 subjects with a heart disease leading to biventricular overload the correlation on VOR of selected QRS amplitude measurements from the Frank, Cube and Tetrahedron lead VCG and from the conventional 12 lead ECG were determined. The measurements selected are listed in Table 12 in the Results section.

RESULTS

Amplitude correlations

The coefficients of correlation between all *the items derived from the Cube and Tetra hedron VCG leads* and the corresponding items derived from the Frank leads are listed in Table 5 The coefficients of correlation between all the items derived from the conventional 12 lead ECG and the corresponding items deri ed from the Frank leads are listed in Table 6

In regard of Cube lead measurements, the highest level of correlation on the corresponding Frank lead measurements wa obtained for Ry *S Sy/(Sy+Ry) S and FRMV (r = 0 917* 0 900 0 878 0 841 and 0 830 respectively) The lowest level of correlation was noted for HRMV RMSV Sx, RMSV/(RMSV+LMSV) and HRMV/(HRMV+HLMV) (r = 0 658 0 667 0 681 0 725 and 0 727 respectively)

In regard of Tetrahedron lead measurements, the highest level of correlation on the corre sponding Frank lead measurements was elicited for Sx/(Sx+Rx) Sx, Ry FRMV and FRMV/(FRMV+FLMV) (r = 0 915 0 935 0 905 0 901 and 0 893 respectively) The lowest level of correlation was noted for Rz/(Rz+Sz) Sy/(Sy+Ry), Rz Sz and HRMV (r = 0 691 0 744 0 75 0 757 and 0 769 respectively)

In regard of conventional 12 lead ECG meas urements, the highest level of correlation on the corresponding Frank lead measurements was found for RaVF RI+SV1+RV6 RV2 RV5 and SaVF/(SaVF+RaVF) (r = 0 909 0 900 0 823 0 778 and 0 762 respectively) The lowest level of correlation was noted for SV5 SV5/(SV5

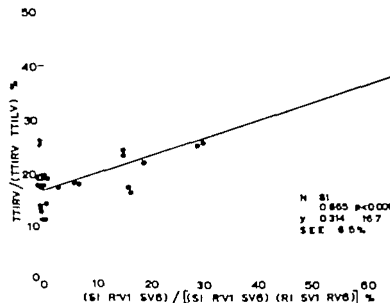


Fig 1 Correlation of (SI RV1 SV6)/((SI RV1 SV6 (RI SV1 RV6)) from conventional ECG leads on VOR the group composed of all 81 subjects in whom both TTIRV and TTILV were determined

Table 5 Coefficients of correlation of all the items derived from Cube and Tetrahedron VCG leads on corresponding items derived from Frank leads.

Measurement	r	
	Cube	Tetrahedron
Sx	0 841	0 933
FRMV	0 850	0 901
HRMV	0 658	0 769
RMSV	0 667	0 779
FRMV+HRMV	0 786	0 874
FLMV	0 816	0 819
HLMV	0 784	0.835
LMSV	0 807	0.851
FLMV+HLMV	0 816	0.855
Sx/(Sx+Rx)	0 744	0 945
Rz/(Rz+Sz)	0 770	0 694
FRMV/(FRMV+FLMV)	0 775	0 893
HRMV/(HRMV+HLMV)	0 727	0 850
RMSV/(RMSV+LMSV)	0 725	0 854
(FRMV+HRMV)/((FRMV+HRMV) +(FLMV+HLMV))	0 764	0 882
Rx	0 681	0 832
Ry	0 947	0 905
Sy	0 900	0 822
Rz	0 755	0 752
Sz	0 790	0 757
Sy/(Sy+Ry)	0 878	0 744

Table 6. Coefficients of correlation of all the items derived from conventional 12 lead ECG on corresponding items derived from Frank leads.

Measurement		r
VCG	ECG	
Sx	SV5	0 685
RMSV	SI+R'V1+SV6	0 712
LMSV	RI+SV1+RV6	0 900
Sx/(Sx+Rx)	SV5/(SV5+RV5)	0 691
Rz/(Rz+Sz)	RV2/(RV2+SV2)	0 747
RMSV/(RMSV+LMSV)	(SI+R'V1+SV6) /((SI+R'V1+SV6)+(RI+SV1+RV6))	0 751
Rx	RV5	0 778
Ry	RaVF	0 909
Sy	SaVF	0 741
Rz	RV2	0 823
Sz	SV2	0 750
Sy/(Sy+Ry)	SaVF/(SaVF+RaVF)	0 762

+RV5) SI+R'V1+SV6 SaVF and RV2/(RV2+SV2) (r = 0 683 0 691 0 712 0 741 and 0.762 respectively).

All correlations were statistically significant ($p < 0.001$)

Prediction of ventricular overload ratio

The coefficients of correlation of the selected items from the Frank Cube and Tetrahedron VCG leads and from the conventional ECG leads on VOR in the group composed of all 81 subjects in whom both TTIRV and TTILV were determined are listed in Table 7 The mean of VOR was 21.9 per cent with a standard deviation of 8 7 per cent

The items with highest correlation on VOR were the quotients (SI+R'V1+SV6)/((SI+R'V1+SV6)+(RI+SV1+RV6)) derived from the conventional ECG leads (Fig 1) Sx/(Sx+Rx) derived from the Frank leads, R,R'V1/(R,R'V1+SV1) derived from the conventional ECG leads, Sx/(Sx+Rx) derived from the Tetrahedron leads and Rz/(Rz+Sz) derived from the Frank leads (r = 0 665 0 652 0 696 0 620 and 0 619 respectively). The lowest level of correlation was observed for the quotients RMSV/(RMSV+LMSV) and Rz/(Rz+Sz) derived from the Cube leads, for HRMV/(HRMV+HLMV) and (FRMV+HRMV)/((FRMV+HRMV)+(FLMV+HLMV)) derived from the Tetrahedron leads and for FRMV/(FRMV+FLMV) derived from the Cube leads (r = 0 435 0 438 0 448, 0 463 and 0 464 respectively)

All of the correlations were statistically significant ($p < 0.001$)

Right ventricular overload

Pressure or volume overload of the right ventricle

The coefficients of correlation between the selected items from the Frank, Cube and Tetrahedron VCG leads and the conventional ECG leads on TTIRV (r1) and VOR (r2) in the 41 subjects with pressure or volume overload of the right ventricle are listed in Table 8 The mean of TTIRV was 740 mmHgsec with a standard deviation of 410 mmHgsec and the mean of VOR was 22.3 per cent with a standard deviation of 7.5 per cent.

The highest level of correlation (Fig 2) was found for the Frank lead items RMSV/(RMSV+LMSV) (r1 = 0 610 and r2 = 0.599) Sx/(Sx+Rx) (r1 = 0.587 and r2 = 0.573) and FRMV/(FRMV+FLMV) (r1 = 0.567) All these correlations were statistically significant ($p < 0.001$) The lowest level of correlation was found for the quotient Rz/(Rz+Sz) derived from the Cube leads (r1 = 0 034 and r2 = 0 180) for the item HRMV derived from the Tetrahedron leads (r1 = 0 163 and r2 = 0.20) and for the item FRMV derived from the Tetrahedron leads (r2 = 0 230) These correlations were not significant ($p > 0.01$)

Pressure overload of the right ventricle

The coefficients of correlation of the selected items from the Frank, Cube and Tetrahedron VCG leads and the conventional ECG leads

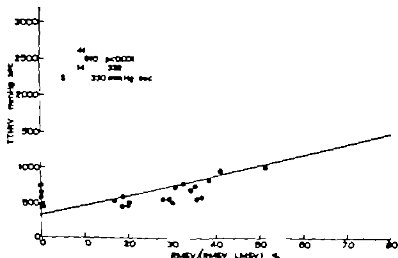


Fig 2 Correlation of RMSV/(RMSV LMSV) from Frank leads on TTIRV in 41 subjects with pressure or volume overload of the right ventricle

Table 7 Coefficients of correlation of the selected items from Frank, Cube and Tetrahedron VCG leads and from conventional ECG lead on VOR in the group composed of all 81 subjects in whom both TTIRV and TTILV were determined

Measurement	Frank	Cube	Tetrahedron	ECG
Sx/(Sx+Rx)	0 652	0 569	0 6?0	—
Rz/(Rz+Sz)	0 612	0 438	0 468	—
FRMV/(FRMV+FLMV)	0 568	0 464	0 470	—
HRMV/(HRMV+HLMV)	0 521	0 562	0 448	—
RMSV/(RMSV+LMSV)	0.564	0 433	0 470	—
(FRMV+HRMV)/((FRMV+HRMV)+(FLMV+HLMV))	0 544	0 509	0 463	—
SI/(SI+RI)	—	—	—	0 601
R,R'V1/(R,R'V1+SV1)	—	—	—	0 6 6
(SI+R'V1+SV6)/((SI+R'V1+SV6)+(RI+SV1+RV6))	—	—	—	0 665

Table 8. Coefficients of correlation of the selected items from Frank Cube and Tetrahedron VCG leads and from conventional ECG lead on TTIRV (r1) and VOR (r2) in 41 subjects with pressure or volume overload of the right ventricle

Measurement	Frank		Cube		Tetrahedron		ECG	
	r1	r2	r1	r2	1	r2	r1	r2
Sx	0 412	0 437	0 379	0 372	0 3 7	0 367	—	—
FRMV	0 410	0 403	0 266	0 291	0 320	0 230	—	—
HRMV	0 321	0 366	0.359	0 339	0 165	0 202	—	—
RMSV	0 442	0 470	0 330	0 306	0 227	0 242	—	—
FRMV+HRMV	0 388	0 408	0.327	0 342	0 287	0 242	—	—
SI	—	—	—	—	—	—	0.314	0 337
R,R'V1	—	—	—	—	—	—	0 328	0 345
SI+R'V1+SV6	—	—	—	—	—	—	0 422	0 436
Sx/(Sx+Rx)	0 387	0 373	0 445	0 466	0 467	0 510	—	—
Rz/(Rz+Sz)	0 269	0 240	0 084	0 180	0 394	0 311	—	—
FRMV/(FRMV+FLMV)	0 567	0 535	0 364	0 404	0 389	0 558	—	—
HRMV/(HRMV+HLMV)	0 502	0 508	0 430	0 455	0.387	0 425	—	—
RMSV/(RMSV+LMSV)	0 610	0 599	0 444	0 447	0 413	0 424	—	—
(FRMV+HRMV)/((FRMV+HRMV)+(FLMV+HLMV))	0 551	0 536	0 402	0 435	0 399	0 395	—	—
SI/(SI+RI)	—	—	—	—	—	—	0 430	0 465
R,R'V1/(R R'V1+SV1)	—	—	—	—	—	—	0.337	0.3?6
(SI+R'V1+SV6)/((SI+R'V1+SV6)+(RI+SV1 RV6))	—	—	—	—	—	—	0 438	0 456

+RV5) SI+R'V1+SV6 SaVF and RV2/(RV2+SV2) (r = 0 683 0 691 0 712 0 741 and 0 762 respectively)

All correlations were statistically significant ($p < 0.001$)

Prediction of ventricular overload ratio

The coefficients of correlation of the selected items from the Frank, Cube and Tetrahedron VCG leads and from the conventional ECG leads on VOR in the group composed of all 81 subjects in whom both TTIRV and TTILV were determined are listed in Table 7 The mean of VOR was 21 9 per cent with a standard deviation of 8 7 per cent.

The items with highest correlation on VOR were the quotients (SI+R'V1+SV6)/((SI+R'V1+SV6)+(RI+SV1+RV6)) derived from the con ventional ECG leads (Fig 1), Sx/(Sx+Rx) derived from the Frank leads, R R'V1/(R,R'V1+SV1) derived from the conventional ECG leads, Sx/(Sx+Rx) derived from the Tetra hedron leads and Rz/(Rz+Sz) derived from the Frank leads (= 0 665 0 652 0 626 0 620 and 0 612, respectively). The lowest level of corre lation was observed for the quotients RMSV/(RMSV+LMSV) and Rz/(Rz+Sz) derived from the Cube leads for HRMV/(HRMV+HLMV) and (FRMV+HRMV)/((FRMV+HRMV)+(FLMV+HLMV)) derived from the Tetrahedron leads and for FRMV/(FRMV+FLMV) derived from the Cube leads (r = 0 433 0 438 0 448 0 463 and 0 464 respectively)

All of the correlations were statistically significant ($p < 0\ 001$)

Right ventricular overload

Pressure or volume overload of the right ventricle

The coefficients of correlation between the selected items from the Frank, Cube and Tetrahedron VCG leads and the conventional ECG leads on TTIRV (r1) and VOR (r2) in the 41 subjects with pressure or volume overload of the right ventricle are listed in Table 8 The mean of TTIRV was 740 mmHgsec with a standard deviation of 410 mmHgsec, and the mean of VOR was 22.3 per cent with a standard deviation of 7.5 per cent.

The highest level of correlation (Fig 2) was found for the Frank lead items RMSV/(RMSV+LMSV) (r1 = 0 610 and r2 = 0 599) Sx/(Sx+Rx) (r1 = 0.587 and r2 = 0.573) and FRMV/(FRMV+FLMV) (r1 = 0 567) All these correlations were statistically significant ($p < 0\ 001$) The lowest level of correlation was found for the quotient Rz/(Rz+Sz) derived from the Cube leads (r1 = 0 084 and r2 = 0 180) for the item HRMV derived from the Tetra hedron leads (r1 = 0 163 and r2 = 0 202) and for the item FRMV derived from the Tetra hedron leads (r2 = 0 230) These correlations were not significant ($p > 0\ 01$)

Pressure overload of the right ventricle

The coefficients of correlation of the selected items from the Frank, Cube and Tetrahedron VCG leads and the conventional ECG leads

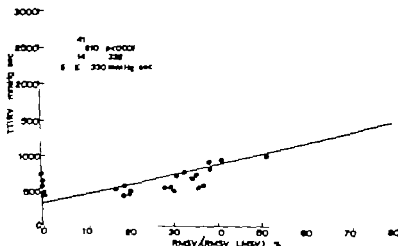


Fig 2. Correlation of RMSV/(RMSV LMSV) from Frank leads on TTIRV in 41 subjects with pressure or volume overload of the right ventricle

Table 7 Coefficients of correlation of the selected [illegible] from Fra[illegible] Tetrahedron VCG lead and from conventional ECG leads on VOR [illegible] composed of all 81 subjects in whom both TTIRV and TTILV [illegible]

Measurement	Frank	Cube	Tet[illegible]	[illegible]
Sx/(Sx+Rx)	0 652	0.569	0 620	
Rz/(Rz+Sz)	0 612	0 435	0 [illegible]	
FRMV/(FRMV+FLMV)	0.568	0 461	0 470	—
HRMV/(HRMV+HLMV)	0 521	0.562	0 [illegible]	—
RMSV/(RMSV+LMSV)	0.564	0 433	0 470	—
(FRMV+HRMV) /((FRMV+HRMV)+(FLMV+HLMV))	0.544	0 509	0 463	—
SI/(SI+RI)	—	—	—	0 601
R,R'V1/(R,R'V1+SV1)	—	—	—	0 6[illegible]
(SI+R'V1+SV6) /((SI+R'V1+SV6)+(RI+SV1+RV6))	—	—	—	0 6[illegible]

Table 8 Coefficients of correlation of the selected items from Frank, Cube and Tetrahedron VCG leads and from [illegible]onventional ECG leads on TTIRV (r1) and VOR (r2) in 41 subjects with pressure o[illegible] [illegible]olume overload of the right [illegible]entricle

Measurement	Frank r1	Frank r2	Cube r1	Cube r2	Tetrahedron r1	Tetrahedron r2	[illegible] r1
Sx	0 412	0 437	0 379	0.372	0.327	0.367	
FRMV	0 410	0 403	0 266	0 291	0.320	0 230	—
HRMV	0 321	0 366	0 359	0 359	0 163	0 202	—
RMSV	0 442	0 470	0 330	0 306	0 227	0 242	—
FRMV+HRMV	0 338	0 403	0 327	0.342	0 287	0 242	—
SI	—	—	—	—	—	—	0.3[illegible]
R,R'V1	—	—	—	—	—	—	0.3[illegible]
SI+R'V1+SV6	—	—	—	—	—	—	0 4[illegible]
Sx/(Sx Rx)	0 587	0 575	0 445	0 466	0 467	0.510	—
Rz/(Rz+Sz)	0 269	0 240	0.084	0 180	0 394	0.311	—
FRMV/(FRMV+FLMV)	0.567	0 535	0 364	0 404	0 389	0 358	—
HRMV/(HRMV+HLMV)	0 502	0 508	0 450	0 453	0 387	0 425	—
RMSV/(RMSV+LMSV)	0 610	0 599	0 444	0 447	0 415	0 42[illegible]	
(FRMV+HRMV) /((FRMV+HRMV)+(FLMV+HLMV))	0 551	0 556	0 402	0 435	0 399	0.35[illegible]	—
SI/(SI+RI)	—	—	—	—	—		
R,R'V1/(R,R'V1+SV1)	—	—	—	—	—		
(SI+R'V1+SV6) /((SI+R'V1+SV6)+(RI+SV1+RV6))	—	—	—	—	—		

Table 9 Coefficients of correlation of the selected items from Frank Cube and Tetrahedron VCG leads and from conventional ECG leads on TTIRV (r1) and VOR (r2) in 17 subjects with pressure overload of the right ventricle

Measurement	Frank		Cube		Tetrahedron		ECG	
	r1	r2	r1	r2	r1	r2	r1	r2
Sx	0.632	0.628	0.536	0.552	0.574	0.590	—	—
FRMV	0.5[illegible]3	0.535	0.343	0.405	0.446	0.431	—	—
HRMV	0.403	0.482	0.523	0.531	0.202	0.242	—	—
RMSV	0.504	0.546	0.535	0.551	0.277	0.289	—	—
FRMV+HRMV	0.485	0.533	0.447	0.489	0.386	0.392	—	—
SI	—	—	—	—	—	—	0.432	0.444
R,R'V1	—	—	—	—	—	—	0.485	0.533
SI+R'V1+SV6	—	—	—	—	—	—	0.718	0.7[illegible]2
Sx/(Sx+Rx)	0.741	0.728	0.604	0.655	0.658	0.660	—	—
Rz/(Rz+Sz)	0.722	0.721	0.395	0.578	0.695	0.649	—	—
FRMV/(FRMV+FLMV)	0.710	0.710	0.542	0.607	0.580	0.587	—	—
HRMV/(HRMV+HLMV)	0.611	0.661	0.601	0.645	0.487	0.495	—	—
RMSV/(RMSV+LMSV)	0.70[illegible]	0.721	0.642	0.677	0.531	0.527	—	—
(FRMV+HRMV) /((FRMV+HRMV)+(FLMV+HLMV))	0.668	0.697	0.581	0.637	0.537	0.546	—	—
SI/(SI+RI)	—	—	—	—	—	—	0.620	0.625
R,R'V1/(R R'V1+SV1)	—	—	—	—	—	—	0.722	0.744
(SI+R'V1+SV6) /((SI+R'V1+SV6)+(RI+SV1+RV6))	—	—	—	—	—	—	0.700	0.704

on TTIRV (r1) and VOR (r2) in the 17 subjects with pressure overload of the right ventricle are listed in Table 9. The mean of TTIRV was 830 mmHgsec with a standard deviation of 590 mmHgsec, and the mean of VOR was 24.1 per cent with a standard deviation of 10.1 per cent.

The highest level of correlation (Fig. 3) was found for the quotients R,R'V1/(R,R'V1+SV1) from the conventional ECG leads (r1 = 0.7[illegible]2 and r2 = 0.744), for Sx/(Sx+Rx) from the Frank lead (r1 = 0.741 and r2 = 0.728) and for Rz/(Rz+Sz) derived from the Frank lead (r1 = 0.722). All these correlations were statistically significant ($p < 0.01$). The lowest level of correlation was found for the items HRMV (r1 = 0.[illegible]0 and r2 = 0.242) and for RMSV (r1 = 0.277 and r2 = 0.289) from the Tetrahedron leads, and for HRMV from the Cube leads (r1 = 0.343). These correlations were not significant ($p > 0.01$).

Volume overload of the right ventricle

The coefficients of correlation of the selected items from the Frank, Cube and Tetrahedron VCG leads and the conventional ECG leads on TTIRV (r1) and VOR (r2) in the 24 subjects with right ventricular volume overload are listed in Table 10. The mean of TTIRV was 680 mmHgsec with a standard deviation of 180 mmHgsec, and the mean of VOR was 21.1 per cent with a standard deviation of 4.7 per cent. No statistically significant correlations were found.

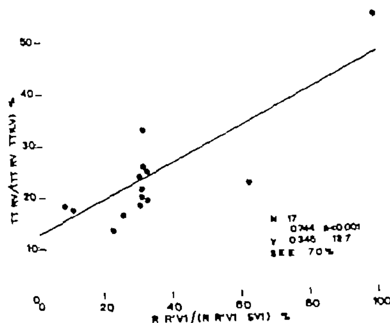


Fig. 3. Correlation of R,R'V1 (R,R'V1 SV1) from conventional ECG leads on VOR in 17 subjects with pressure overload of the right ventricle.

Table 10. Coefficients of correlation of the selected items from Frank Cube and Tetrahedron VCG leads and from conventional ECG leads o TTIRV (r1) and VOR (r2) in 24 subjects with right entricular olume overload.

Measurement	Frank		Cube		Tetrahedron		ECG	
	1	r2	1	2	r1	r2	r1	r2
Sx	0.412	0.474	0.415	0.328	0.254	0.345	—	—
FRMV	0.542	0.502	0.414	0.319	0.150	0.114	—	—
HRMV	0.186	0.177	0.411	0.356	0.091	0.147	—	—
RMSV	0.442	0.417	0.429	0.281	0.208	0.227	—	—
FRMV+HRMV	0.284	0.258	0.441	0.360	0.128	0.006	0.280	0.351
SI	—	—	—	—	—	—	0.032	0.092
R,R'V1	—	—	—	—	—	—	0.417	0.438
SI+R'V1+SV6	—	—	—	—	—	—	—	—
Sx/(Sx+Rx)	0.357	0.474	0.346	0.334	0.248	0.478	—	—
Rz/(Rz+Sz)	–0.498	–0.322	–0.191	–0.0 2	–0.162	–0.191	—	—
FRMV/(FRMV+FLMV)	0.414	0.364	0.366	0.364	0.271	0.211	—	—
HRMV/(HRMV+HLMV)	0.136	0.140	0.360	0.374	0.218	0.415	—	—
RMSV/(RMSV+LMSV)	0.469	0.450	0.402	0.340	0.258	0.400		
(FRMV+HRMV) /((FRMV+HRMV)+(FLMV+HLMV))	0.301	0.259	0.374	0.375	0.254	0.317	—	—
SI/(SI+RI)	—	—	—	—	—	—	0.205	0.443
R,R'V1/(R,R'V1+SV1)	—	—	—	—	—	—	–0.061	–0.001
(SI+R'V1+SV6) /((SI+R'V1+SV6)+(RI+SV1+RV6))	—	—	—	—	—	—	0.351	0.469

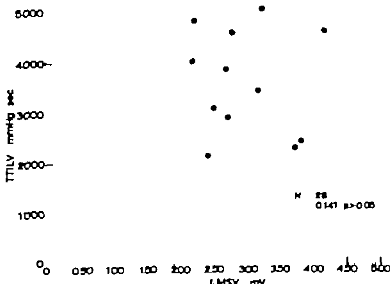


Fig 4 LMSV from Frank lead plotted against TTILV in 28 subjec with lef entricular overload.

Left ventricular overload

The coefficients of correlation of the selected items from the Frank, Cube and Tetrahedron VCG leads and the conventional ECG leads on TTILV in the 28 subjects with left ventricular overload are listed in Table 11 The mean of TTILV was 3760 mmHgsec with a standard deviation of 1080 mmHgsec. No statistically significant correlations were found. As a representative result the LMSV meas urements from the Frank leads has been plotted against TTILV in Fig 4 illustrating lack of any significant correlation

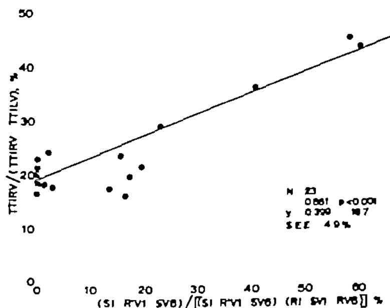


Fig 5 Correlation of SI R I SV6 SI RV1 SV6 +(RI SV1 RV6 from conventional ECG leads on VOR in 23 subjects with ... lar overload

Table 11 Coefficients of correlation of the selected items from Frank, Cube and Tetrahedron VCG leads and from conventional ECG leads on TTILV in 28 subjects with left ventricular overload

Measurement	r			
	Frank	Cube	Tetrahedron	ECG
FLMV	0 129	0 108	0 050	—
HLMV	0 209	0 145	0 214	—
LMSV	0 141	0 077	0 112	—
FLMV+HLMV	0 182	0 155	0 146	—
RI+SV1	—	—	—	0 370
RI+R,SaVF	—	—	—	0 15
SV1+RV5 6	—	—	—	0 197
SV1+R,SaVF	—	—	—	0 13
RI+SV1+RV6	—	—	—	0 411
RI+R,SaVF+SV1	—	—	—	0 67

Table 12. Coefficients of correlation of the selected items from Frank, Cube and Tetrahedron VCG leads and from conventional ECG leads on VOR in 25 subjects with biventricular overload.

Measurement	r			
	Frank	Cube	Tetrahedron	ECG
Sx/(Sx+Rx)	0 836	0.568	0 781	—
Rz/(Rz+Sz)	0 864	0 737	0 723	—
FRMV/(FRMV+FLMV)	0 705	0 402	0 700	—
HRMV/(HRMV+HLMV)	0 580	0 587	0 526	—
RMSV/(RMSV+LMSV)	0.555	0 235	0 606	—
(FRMV+HRMV)/((FRMV+HRMV)+(FLMV+HLMV))	0 626	0 476	0 618	—
SI/(SI+RI)	—	—	—	0 784
R,R'V1/(R R'V1+SV1)	—	—	—	0.858
(SI R'V1+SV6)/((SI+R'V1+SV6)+(RI+SV1+RV6))	—	—	—	0 881

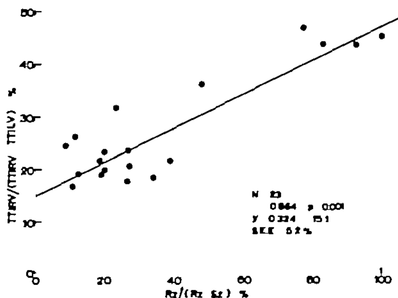


Fig 6 Correlation of Rz/(Rz Sz) from Frank leads on VOR in 23 subjects ith biventricular overload

Biventricular overload

The coefficients of correlation of the selected items from the Frank, Cube and Tetrahedron VCG leads and the conventional ECG leads on VOR in the 23 subjects with biventricula overload are listed in Table 12. The mean of VOR was 21 9 per cent with a standard deviation of 8 7 per cent.

The highest level of correlation was found for the quotients (SI+R'V1+SV6)/((SI+R'V1+SV6)+(RI+SV1+RV6)) from the conventional ECG leads (Fig 5), for Rz/(Rz+Sz) from the Frank leads (Fig 6) for R,R'V1/(R,R'V1+SV1) from the conventional ECG leads (Fig 7), for Sx/(Sx+Rx) from the Frank leads (Fig 8) and for SI/(SI+RI) from the conventional ECG leads (r = 0 881 0.864 0 858 0 836 and

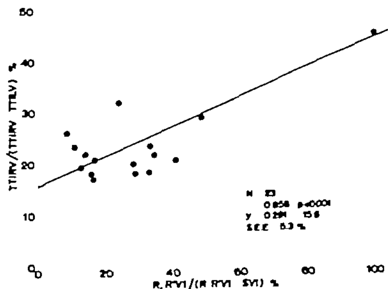


Fig 7 Correlation of R,R'V1/ R,R'V1 SV1 from convent onal ECG lead on VOR in 23 subjects with biventricular overload

0.784 respectively) All these correlations were statistically significant ($p < 0.001$). The lowest level of correlation was found for the quotients RMSV/(RMSV+LMSV), FRMV/(FRMV+FLMV) and (FRMV+HRMV)/((FRMV+HRMV)+(FLMV+HLMV)) from the Cube leads, for HRMV/(HRMV+HLMV) from the Tetrahedron leads and for RMSV/(RMSV+LMSV) from the Frank leads ($r = 0.235$, 0.402, 0.467, 0.526 and 0.555 respectively). All these correlations except that of RMSV/(RMSV+LMSV) from the Frank leads lacked statistical significance ($p > 0.01$).

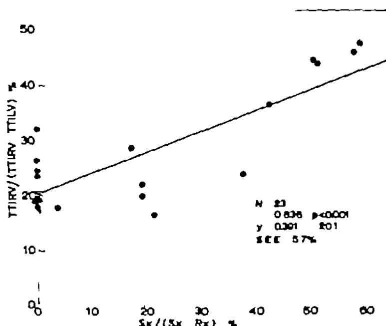


Fig. 8. Correlation of Sx/(Sx+Rx) from Frank leads on VOR in 23 subjects with biventricular overload

be responsible for the low correlations in the present study

1 The subjects with pressure and volume overload were combined to form one composite group because only relatively few patients in the material had "pure pressure or volume overload

2 The presence of wide variation of QRS amplitudes produced by individual constitution al factors (Grant 1956 Selzer et al 1958 Kilty and Lepeschkin 1965 Hirsch 1966 and P pberger et al 1967) includ ng factors such as non homogeneity of body tissues (Brody 1956 Bayley and Berry 1963 Bayley and Berry 1966 Grayzel and Lizzi 1967 McFee and Rush 1967 McFee and Rush 1968 and Bayley et al 1969) On the other hand one would expect these same factors to influence the correlations also in right ventricular overload.

Reasons which may account for the re latively high correlations between selected QRS amplitude measurements and certain hemo dynamic parameters, reported by Hugenholtz and Gamboa (1964) Gamboa et al (1965) and Postell et al (1969) in subjects with left ventricular overload, are the facts that only subjects with left ventricular pressure overload were studied and that the individual constitutional variations may have been smaller in the groups in question than among the subjects of the present study

In the 23 subjects with biventricular over load statistically significant correlations were found between many of the selected ECG and VCG measurements, and VOR. A relatively high level of correlation on VOR was found for the quotients (S1+R'V1+SV6)/((S1+R'V1+SV6)+(R1+SV1+RV6)) from the conventional ECG leads (Fig 5) for Rz/(Rz+Sz) from the Frank leads (Fig 6) for R,R'V1/(R,R'V1+SV1) from the conventional ECG leads (Fig 7) for Sx/(Sx+Rx) from the Frank leads (Fig 8) and for S1/(S1+R1) from the con entional ECG leads (r = 0 881 0 864 0 858 0 836 and 0 784 respectively) S E.E. for the best correl tion was 4 9 per cent

These finding suggest that selected QRS amplitude measurements have at least po tentially practical gnificance in p edicti n of the severity of entricular overload, and partu cula ly of the overload ratio in heart lesions which may produce biventricular overload One possible reason for the better correlations of the same ECG and VCG measurements on VOR in subjects with biventricular overload than in those with right ventricular overload is that the values of VOR in biventricular overload cover a wider range than those in right ventricular overload In subjects with ventricular septal defect the R/S ratio and the R wave amplitude in lead V1 have been reported to present a tendency to increase with increasing right ventricular systolic peak pressure (Burch and DePasquale 1960 and Papadopoulos et al 1965) The R wave amplitude in lead V1 has also been reported to increase with increasing ratio of right ventricular systolic pressure to systemic systolic peak pressure and in regard of the R wave amplitudes in lead V5 and V6 an tendency to increase with increasing volume of the left to-right shunt has been reported (Hubbard and Angle 1957) However to the author s best knowledge no quantitative correlation between ECG and VCG amplitudes and any particular hemodynamic parameter has been previously reported at least, not in adult subjects with biventricular overload or with a heart defect potentially producing biventricular overload

Among the five items which presented the highest level of correlation on VOR in the subjects with biventricular overload were the quotients Rz/(Rz+Sz) and Sx/(Sx+Rx) derived from the Frank leads (r = 0 864 and 0 836, respectively) The quotient Rz/Rz+Sz) from the Frank leads had the lowest level of correlation on the corresponding Tetrahedron lead item in respect of amplitude (r = 0.694). The level of correlation on VOR was lower though not very markedly for the quotient Rz/(Rz+Sz) from the Tetrahedron leads than that of the corresponding Frank lead item (r = 0 723 and 0 864 respectively) The quotient Sx/(Sx+Rx) from the Frank leads presented the highest level of correlation on the corresponding Tetrahedron lead item in respect of amplitude (r = 0 945) The level of corre lation on VOR was also lower for the quotient Sx/(Sx+Rx) from the Tetrahedron lead than that of the corresponding Frank lead tem (= 0 781 and 0 836 respectively)

In analysis of relationships between hemodynamic parameters and ECG or VCG amplitudes, the highest levels of correlation on VOR or TTIRV were systematically obtained for items derived from the conventional 12 lead ECG or from the Frank lead VCG. The lowest correlations in corresponding comparisons were obtained for the Cube or Tetrahedron lead items. It appears that some of the criteria derived from the conventional 12-lead ECG perform equally well as the measurements made from the orthogonal lead system. On the other hand it should be noted that the Frank lead parameters with best correlations were simpler as a rule than the conventional ECG items. In prediction of VOR, for instance, the best conventional ECG item (r = 0 665) was the quotient (SI+R'VI+SV6)/((SI+R'V1+SV6)+(RI+SV1+RV6)) and the best Frank lead item (r = 0 652) was the quotient Sx/(Sx+Rx).

It should also be emphasized that the differences in level of correlation between different lead system were relatively minor and no lead system can be definitely recommended as superior to the other systems on the basis of the limited analysis of the present study.

The ECG and VCG measurements used in the present study are perhaps not the best and most discriminating variables for correlation on hemodynamic parameters. It is likely that better results can be obtained by means of automatic computer measurements and multivariate analysis of the relationships. It should be noted, however that most of the variables chosen for the present study are commonly accepted and used in ectorcardiographic studies and that they are suitable for usual analysis.

Although theoretically superior the corrected lead system may still be too sensitive to marked individual constitutional variations. It is the authors impression that the results of the present study have been significantly influenced by both factors just mentioned.

Limitations of the present study

Clinical material like that used in the present study are selected owing to various practical reason. This fact may have had some influence on the correlations of ECG and VCG measurements on hemodynamic parameters which were found in the present study.

Rubbing of the skin under the electrodes perhaps does not completely eliminate possible amplitude distortions produced by high skin electrode interface resistance (Frank 1956 and Spach et al 1966). It is unlikely however that this factor would have significantly influenced the measurements in the present study.

Errors arising from possible displacements of the electrodes (Simonson et al 1966 b) cannot be ruled out completely. Careful attention was paid to exact placement of the electrodes in the present study in order to reduce the likelihood of such errors.

Quiet resting respiration during recording was allowed in the present study. This may cause minor variations in the QRS amplitudes (Simonson et al. 1957 Beswick and Jordan 1961 and Flaherty et al. 1967). Breath holding was not attempted in the present study because of its inconvenience when multiple records were required.

Variation from beat to beat is certain to cause some variation in the QRS amplitudes (Fischmann et al. 1968). The errors introduced by this variation are reduced but not entirely eliminated, when measurements are made from two beats, as was done in the present study. Without tape recording, however measurements from a great number of beats would have presented a highly cumbersome task.

Errors in definition and identification of the so called "zero" reference point may cause some distortion in the measured QRS amplitudes especially when the recorded amplitudes were small, as was the case with some records with Cube and Tetrahedron leads. It is unlikely however that this error would have had any significant systematic influence on the results from the practical point of view.

The ECG and VCG measurements used in the present study are of course rather arbitrary. They were selected because they have been empirically observed to present a tendency of change in ventricular hypertrophy. It should also be noted that corresponding measurements from the different leads are not

necessarily identical in the electrophysiological sense. This may have biased the results in favour or against of the lead systems used. The items from the conventional ECG leads chosen in the present study were selected in an effort to add to the correlation analysis some items which can be expected to correlate well with the corresponding measurements from the VCG leads. They are of course not directly comparable with the VCG lead measurements. It is also possible that other measurements, such as the orientation angles of instantaneous vectors, may posses a better level of correlation on hemodynamic parameters than that of the amplitude measurements (Witham and McDaniel 1970).

The correlation of TTI on the oxygen consumption of the heart is perhaps not very good in some circumstances (Kühn and Brachfeld 1969). TTI also reflects primarily the ventricular pressure as a function of time and thus may have biased the results in favour of the subject group with pressure overload only. The advantage of TTI is, however, that it is easy to measure.

The tension time per beat was measured from three representative beats in most subjects of the present study and from two beats in a few subjects only. The use of so few heart beats may well cause some errors in TTI, but measurements from a greater number of beats were precluded by practical limitations.

The tension time per beat of the left ventricle was measured from the brachial artery pressure curve in some subjects. This probably does not cause any significant errors (Rowell et al. 1968).

No attempt was made in this study to consider the effect of digitalis on myocardial oxygen consumption (Mason and Braunwald 1968 and Sonnenblick et al. 1965) and its possible influence on the results obtained.

It is likely that the combination of all these possible errors and variations in ECG and VCG and hemodynamic measurements, in the present study has reduced the level of the observed correlations between ECG and VCG measurements and hemodynamic parameters.

SUMMARY

The predictability of hemodynamic over load and overload ratio of the entricles from selected QRS amplitudes measured from the Frank, Cube and Tetrahed on VCG leads and from the conventional ECG leads was tudied in 41 subjects w th a heart disease leading to right ventricular overload, in 28 subjects with a heart disease leading to left entricular overload and in 23 subjects with a heart disease leading to biventricular o erload. The entricular overload ratio (VOR) was defined as the ratio TTIRV/(TTIRV+TTILV) where TTIRV and TTILV are the right and the left ventricular tension time index, respectively

The correlations between selected QRS amplitude measurements from the Cube and Tetrahedron VCG leads and from the convent ional ECG leads on the correspond ng measure ments from the Frank VCG leads were studied in 137 clin cally abnormal subject with a wide spectrum of different types of hemodynamic overload of the entricles

The most important findings are

1 Significant orrelations were found between several of the ECG and VCG measurement used as ndices for the entricular p epond erance ratio (VPR) and VOR n the group of 81 subjects in whom both TTIRV and TTILV were determined The best correlation was found for the quotient (SI+R'V1+SV6)/((SI R'V1+SV6)+(RI+SV1+RV6)) derived from the conventional ECG lead (r = 0 665 p < 0 001 = S E.E. = 6 5 per cent) These findings suggest that although only relatively inaccurate prediction of VOR can be mad f om the ECG and VCG measurements chosen in the present tudy VOR ha some dir ct or ind ect nfluen e on the QRS amplitudes, rega dles of the type of entricula loading

2 A relatively high orrelation (r = 0 881 p < 0 001 S E.E. = 4 9 per cent) was ob served between the quotient (SI+R'V1+SV6) /((SI+R'V1+SV6)+(RI+SV1+RV6)) derived from the conventional ECG lead and VOR in the 23 subjects with a heart disease leading to bi entricular overload. About the same level of correlation was found for the quotients Rz/(Rz+Sz) measured from the Frank leads for R,R V1/(R,R'V1+SV1) from the onventional ECG leads and fo Sx/(Sx+Rx) from the Frank leads (r = 0 864 0 858 and 0 836 e spectively) These findings uggest that at least approximate pred ction of VOR is possibl from ECG and VCG measurements in subjects with a heart disease leading to bivent icula overload

3 Significant, though not high, correlations wer found between most of the ECG and VCG measurements selected and the hemo dynamic parameters in the 41 subjects with right ventricular p essure o volume overload The best correlation was found between the quotient RMSV/(RMSV+LMSV) derived f om the Frank leads and TTIRV (= 0 610 p < 0 001 S E.E. = 330 mmHgsec) Better corre lat ons were observed in the 17 ubject with pressure o erload of the right entricle only The best orrelation was found between VOR and the quotient R R'V1/(R,R'V1+SV1) derived from the conventional ECG leads (= 0 744 p < 0 001 S F E = 7 0 per cent) In the 24 subjects w th right ventricula volume overload no statistically sign ficant orrelations were found.

4 No statistically significant correlations were found between any of the selected ECG and VCG measurements nd hemodynamic para meters in the 28 ubjects with left ventricular overload

5 The differences in level of correlation on hemodynamic parameters of the items derived from different lead systems were relatively small and no lead system can be definitely recommended as superior to the other systems on the basis of the present study

6 Statistically significant correlations were found between all the selected items from the Cube and Tetrahedron VCG leads and from the conventional ECG leads, and the corresponding measurements from the Frank VCG leads. The correlations were relatively high for Ry derived from the Cube leads, for Sx/(Sx+Rx) and Sx from the Tetrahedron leads, for RaVF from the conventional ECG leads and for Ry from the Tetrahedron leads ($r = 0.947$ 0 945 0 955 0 909 and 0 901 respectively) Of these items, however only one ((Sx/(S +Rx)) was among those used in the studies of correlation between QRS amplitude measurements and hemodynamic parameters.

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Physical Performance and Hematological Parameters

With special reference to hemoglobin and maximal oxygen uptake

By ODD D VELLAR
and LARS HERMANSEN

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Physical Performance and
Hematological Parameters

FROM THE INSTITUTE OF HYGIENE, UNIVERSITY OF OSLO AND
THE INSTITUTE OF WORK PHYSIOLOGY OSLO NORWAY

Physical Performance and Hematological Parameters

with special reference to hemoglobin and maximal oxygen uptake

By

ODD D VELLAR and

LARS HERMANSEN

To

KÅRE RODAHL

Professor of Physiology

CONTENTS

PREFACE

This publication is based on a manuscript which was submitted to the University of Oslo in February 1970 and which was awarded His Majesty's Gold Medal 1969/70. The manuscript, however, has subsequently been revised.

The authors would like to express thanks to Professor dr. med. Haakon Natvig, head of the Institute of Hygiene, University of Oslo, and Professor dr. med. Kåre Rodahl, head of the Institute of Work Physiology, for their constant interest and support in this study.

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INTRODUCTION

The individual's *physical performance* is determined by several factors [7]

Energy liberation
aerobic processes
anaerobic processes

Neuro-muscular function
muscle strength
technique (co-ordination)

Psychological factors
motivation

Depending of the nature of the activity and the duration and intensity of the work the relative importance of the above mentioned factors may vary However during prolonged severe exercise or during ordinary daily activities, *aerobic processes* play the most important role. Assessment of the individual's *oxygen uptake* during exercise is a measure of the amount of energy liberated aerobically since one liter of oxygen consumed corresponds to approximately 5 kcal. Consequently the capacity to perform heavy muscular exercise depends on the individual's ability to transport oxygen from the lungs to active tissues.

Systemic oxygen transport (SO T) may be defined as the amount of oxygen delivered to the tissues by the systemic circulation per unit time. It is determined by the systemic blood flow (cardiac output, Q) and the arterial oxygen content (a0) and may be calculated from the equation

$$SO\ T\ (ml\ O_2/min) =$$
$$Q\ (ml/min) \times aO_2\ (ml\ O_2/ml)$$

Normally 97 per cent of the oxygen transported is carried in chemical combination with *hemoglobin* in the red blood cells (1 g hemoglobin is able to transport 1.34 ml of oxygen) Thus, an increase (polycythemia) or a decrease (anemia) in the hemoglobin concentration would change the oxygen carrying capacity of the blood.

Nutritional anemias, particularly due to iron deficiency are highly prevalent in both developed and undeveloped countries [16,38] However we are not aware of any valid epidemiological evidence showing that mild or moderate anemia has any harmful effects. On the contrary a reduced level of hemoglobin might have a beneficial effect on the health condition as it is associated with reduced levels of serum-cholesterol and other blood lipids [25 42] Whether this association is causal or not is still an open question. It should also be noted that the viscosity of the circulating blood is lowered in anemia. These factors might explain, at least partly the supposed lower risk of cardiovascular disease in anemic subjects [14, 17 20 22]

The symptoms usually ascribed to anemia are often vague and difficult to evaluate. Elwood et al. [24, 26, 27 58] have examined this problem in a number of community-based studies, but failed to obtain any convincing evidence of a significant association between the hemoglobin level in women and symptoms such as fatigue, breathlessness, palpitations, dizziness, headache and irritability Although iron therapy increased the hemoglobin level considerably placebo medication gave a comparable improvement in wellbeing and symptoms. Consequently the authors concluded that it is the 'neurotic' rather than the 'anemic women who complain of the symptoms examined. The findings of Elwood et al. have been confirmed by Dawson & Ogston [21] in hospitalized patients. A history of recent onset of facial pallor was the only important symptom of anemia *per se*. Thus, there is no clear evidence which could substantiate the clinical concept of moderate iron-deficiency anemia as a disease with commonly recognized symptoms.

However since hemoglobin is the main oxygen carrier of the blood and oxygen uptake is of primary importance for energy liberation during work, it seems reasonable to postulate that changes i hemoglobin concentration might influence physical per formance. Several investigators [2, 11 13, 15 19 32, 43, 51, 5?] have examined this problem, but the studies have yielded conflicting results which are difficult to compare, partly due to differences in the material examined, and partly due to vari ations in the methods employed for the estimation of physical performance.

The object of the present study was to evaluate the effect of variations in the hemoglobin level (and related hematologi cal parameters) on the individual s physical performance measured by his *maximal oxygen uptake*. This particular method was chosen as it gives valuable information about both maximal work power and the functional capacity of the oxygen transport system. Since measurements of maximal oxygen uptake require considerable motiv ation and almost maximal effort from the subjects, only young and healthy individuals were examined.

In order to study subjects with pronounced variations in both maximal oxygen uptake and hemoglobin levels, school children and trained students of both sexes were investigated. A few clinical cases of iron-deficiency anemia, who were otherwise in good health, were also included.

The aim of the study was also to examine the effect of *iron supplements* on the assumed relationship between the oxygen carrying capacity of the blood and the maximal oxygen uptake. Therefore, long term iron and placebo medication was given to the students and the clinical cases of anemia.

MATERIAL

A. PRIMARY MATERIAL

Altogether 280 individuals were included in the primary material. Of these, 97 were students and 183 schoolchildren (Table I)

Students

This investigation was carried out from Aug. 1967 to May 1968 and included 97 physical education students from Oslo. The mean age of the 47 men was 24.5 years and of the women 22.4 years.

The general health condition of the students was good. Not all individuals, however were necessarily iron-repleted. According to the criteria of normal hematological values in Norwegian adult men and women as suggested by Natvig & Vellar [39] the students were divided into two groups

a) with normal Hb-values

b) with sub-normal Hb-values.

This grouping was based on the results of the first hematological examination in Aug. 1967

By paired-sampling technique all individuals with normal Hb-values (group a) were allocated to two subgroups

1) the iron therapy group

2) the placebo group.

Table I Composition of the primary material (students and schoolchildren)

Groups	Men		Women		Total	
Students	47		50		97	
Schoolchildren						
Initial material	39	95	33	88	72	183
Additional material	56		55		111	
Total	142		138		280	

All individuals with sub-normal Hb-values (group b) were also given iron according to the same scheme as used for the normal individuals iron tablets in the form of ferrofumarate. One iron tablet was equivalent to approximately 60 mg of bivalent iron. The iron tablets and the placebo tablets were of identical shape and size and labelled "ferrofumarate X" and "ferrofumarate Y" respectively supplied by Nyegaard & Co Oslo.

Each person received the tablets packed in boxes of 100 and was instructed to take one tablet once a day regardless of the blood values obtained at the first examination. The tablets were taken in the evening. Thus, the blood samples were taken before the intake of the dose on the day of each examination.

Re-examinations were performed with approximately eight weeks' intervals, the last one in May 1968. Including the initial examination, six examinations were undertaken altogether At the end of the experimental period (40 weeks) the actual consumption of iron and placebo tablets was recorded.

The various groups and subgroups of the students as well as the various steps of the investigation are presented in Fig 1.

Schoolchildren

The 183 schoolchildren examined were pupils at two elementary schools in the county of Finnmark. The first examination was carried out in Oct. 1968 on 72 schoolchildren, 39 boys and 33 girls (*the initial material*) as shown in Table 1.

An *additional material* of 56 boys and 55 girls was included in the final examination which was performed in April 1969 The various groups of the schoolchildren as

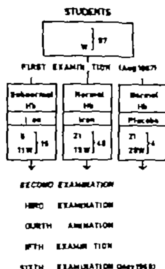


Fig. 1. The various steps of the investigation of hematological indices and physical work capacity in students (M = men, and W = women)



Fig. 2. The various steps of the investigation of hematological indices and physical work capacity in schoolchildren (B = boys, and G = girls)

well as the various steps of the investigation are presented in Fig. 2. The sex and age distribution of the *final material* of the school-children examined is shown in Table II.

B. THE SUPPLEMENTARY MATERIAL OF FEMALE STUDENTS WITH LOW NORMAL/SUB-NORMAL Hb-VALUES

A supplementary material of physical education students was examined during the period Aug.—Oct. 1969. Altogether 68 female students were screened with a hemoglobin determination which gave a mean concentration of 13.55 g/100 ml (SD = 0.86).

Eight subjects with low-normal (12.6 — 13.0 g/100 ml) or sub-normal (below 12.5 g/100 ml) hemoglobin values were selected for the follow up examination (Table III). The remainder 60 students, will not be considered in the following. The hematological indices and the physical work capacity were determined with weekly intervals for seven weeks. Iron tablets were adminis-

Table II. Sex and age distribution of the final material of schoolchildren examined

Age (yrs)	Boys	Girls	Total
10	9	5	14
11	8	11	19
12	23	30	53
13	31	23	54
14	10	7	17
15	10	5	15
16	4	7	11
Total	95	88	183

Table III. The supplementary material of female students with low-normal or sub-normal Hb-values

Group	No in group	Hb (g/100 ml) at start (mean)	Treatment Weeks 1–4	Weeks 5–7
Low-normal	2	12.9	Iron	Iron
	2	13.0	Placebo	Iron
Sub-normal		12.1	Iron	Iron
	2	1_.2	Placebo	Iron
Total	8	12.5		

tered to half the subjects in both the low normal and the sub-normal groups. The other half of the material was given placebo tablets during the last three weeks of the experiment. The dose was usually three tablets a day. On the day of each examination no tablets were taken before the blood was drawn. Otherwise, the scheme of administration was similar to that described for the primary material.

C. SELECTED CASES OF IRON DEFICIENCY ANEMIA

During the period March—Dec. 1969 four cases of iron-deficiency anemia were found suitable for examination of the physical work capacity. The description of these anemic cases is given in Table IV.

The hematological indices and the physical work capacity were determined for periods of 7 to 26 weeks. The interval between the re-examinations was usually about one to two weeks but varied from 3 to 44 days. None of the subjects donated blood during the period of investigation. Iron tablets were administered to three of the subjects, the dose was 2—3 tablets a day. The fourth subject received placebo tablets during the first four weeks of the follow-up period. From then on, however the placebo tablets were replaced by iron tablets. Otheriwse, the scheme of administration was similar to that described for the students.

Table IV. Selected cases of iron-deficiency anemia

Subject	Sex	Age (yrs)	Hb (g/100 ml) at start	Follow-up period (weeks)	Treatment
T.H. blood donor	male	40	14.2	13	Iron
T.H. private prac. patient	female	33	10.9	7	Iron
R.L. blood donor	female	35	11.0	20	Iron
S.H. blood donor	female	18	11.4	26	placebo (weeks 1–4) iron (weeks 5–26)

METHODS

A. MEASUREMENT OF HEMATOLOGICAL INDICES

The following parameters were determined hemoglobin concentration (Hb) hematocrit (Hct) mean corpuscular hemoglobin concentration (MCHC) serum iron, total iron binding capacity (TIBC) and percent saturation of transferrin (transferrin sat %) In schoolchildren, however only Hb, Hct and MCHC were recorded.

In 10 male and 10 female students of the primary material the total quantity of hemoglobin (THb) was also estimated at the first examination. The estimation was repeated at the sixth examination in the same subjects and with the addition of three men and one woman.

Hb Hct and MCHC

The Hb determinations were performed by the cyanmethemoglobin method with photoelectric reading in a Linson Junior photoelectric colorimeter The colorimeter was calibrated against standardized cyanmethemoglobin solutions. The Hct was measured in heparinized capillary tubes after centrifugation in a hematocrit centrifuge (AB L. Ljungberg & Co., Stockholm) From the recorded values of Hb and Hct, the MCHC was calculated.

The details of the methodology are consistent with the standard procedure used by Natvig et al. [38] in the comprehensive study of the hemoglobin values in Norway

Routinely the determinations of Hb and Hct were performed in blood samples collected in heparinized centrifuge tubes after venepuncture of the antecubital vein. In the schoolchildren, however the determinations were performed in blood samples taken from a prewarmed fingertip, by pricking with lancets.

The blood samples from the students of the primary material (A) and the supplementary material (B) were obtained in the afternoon. In the remainder of the material the blood sampling usually was performed earlier in the day

Serum iron TIBC and percent saturation of transferrin

The Teepol-bathophenanthroline method modified by Aakevold & Vellar [3] was used for the determination of serum iron. The TIBC was measured by the method described by Ramsay [41] although the final step was modified and adopted to the Teepol-bathopenanthroline procedure for the serum iron determination. From the measured values of serum iron and TIBC, the transferrin sat% was calculated (serum iron as a percentage of TIBC)

The total quantity of hemoglobin

The total quantity of hemoglobin (THb) was calculated from estimations of Hb concentration and blood volume. The blood volume was calculated from Hct and the plasma volume which was measured according to the radio-iodine plasma protein method of Williams & Fine [57] Ten ml of an isotonic NaCl-solution, containing 1.2 % albumin labeled with ^{131}J and with an activity of 0.3 μCi per ml, was injected intravenously and the degree of dilution was taken as a measure of the plasma volume of the subject. After a period of 10 min, allowing a thorough mixing between the injected albumin J and the plasma protein pool, a blood sample of 15 ml was withdrawn into a heparinized syringe. The blood was centrifuged and the isotopic measure-

ments performed in an aliquot of 5 ml of plasma, by using a Frieslke-Hoephner scin tillation counter

B. ASSESSMENT OF PHYSICAL WORK CAPACITY

Maximal oxygen uptake constitutes the most commonly used index of the physical work capacity In the present investigation, two different methods are used to measure the maximal oxygen uptake, viz. the *indirect* and the *direct* method. The values are given as liter/min, and also corrected for variations in body weight (ml kg x min) As the direct method requires considerable motivation and cooperation even in young subjects, and is time-consuming this method has neither been employed to test all subjects, nor used in all examinations of the same subject.

Maximal oxygen uptake (indirect method)

In all subjects maximal oxygen uptake was calculated from measurements of heart rate and work load according to the method described by Astrand & Ryhming [8] The experiments were performed on a mechanically braked bicycle ergometer described by von Döbeln [54] The height of the saddle on the bicycle ergometer was adjusted to each individual to ensure a slight bending of the knee when the anterior part of the foot was placed on the pedal in its lowest position. The pedal rate was kept constant at 50 revolutions per min by using a conventional metronome.

Maximal oxygen uptake (direct method)

In most subjects maximal oxygen uptake was also determined directly by using the Douglas bag method. (It was performed in all schoolchildren, in a sample of 41 students of the primary material at the first examination and in 31 students at the sixth examination, in the supplementary material of female students and in the anemic cases) In general, the procedure suggested by Hermansen & Saltin [31] was used in all determinations. The subjects were running uphill on a motordriven treadmill at a speed which lead to exhaustion within 4—6 minutes. The inclination of the treadmill was commonly kept at 3 (5.25 %)

All maximal experiments on the treadmill startet with 10 minutes warming up on a work load which represented 50—70 % of the maximal oxygen uptake of the individual. The expired air was collected in a series of Douglas bags during the last minutes of the maximal exercise, until the subject was completely exhausted. The collected volume of the expired air was measured in a spirometer and gas analysis was performed on a Scholander apparatus [49]

The respiratory valve had a dead space of 100 ml. The inner diameter of the valve, the stopcock and the tube of the Douglas bags was 30 mm. The connecting tubes were smooth and not corrugated, with an inner diameter of 35 mm. The length of the connecting tube, from the subject to the bag, was approximately 50 cm.

Heart rate was continuously recorded during the air collection period by using a conventional electrocardiogram apparatus.

Blood lactic acid concentration during the first 5—10 minutes after the maximal run was analyzed according to the Strom modification of the colorimetric method of Barker & Summerson [53]

The data for heart rate and blood lactic acid, however are not presented in the results, but are included as supporting criteria in the evaluation of the maximal oxygen uptake measurements, as proposed by Astrand [5]

C. STATISTICAL ANALYSIS

The arithmetric mean ($\bar{x}$) the standard deviation (SD) and the standard error of the mean (SE) have been calculated according to standard statistical methods.

METHODS

A. MEASUREMENT OF HEMATOLOGICAL INDICES

The following parameters were determined hemoglobin concentration (Hb) hematocrit (Hct) mean corpuscular hemoglobin con centration (MCHC) serum iron, total iron binding capacity (TIBC) and percent saturation of transferrin (transferrin sat %) In schoolchildren, however only Hb, Hct and MCHC were recorded.

In 10 male and 10 female students of the primary material the total quantity of hemoglobin (THb) was also estimated at the first examination. The estimation was repeated at the sixth examination in the same subjects and with the addition of three men and one woman.

Hb Hct and MCHC

The Hb determinations were performed by the cyanmethemoglobin method with photoelectric reading in a Linson Junior photoelectric colorimeter The colorimeter was calibrated against standardized cyanmethemoglobin solutions. The Hct was measured in heparinized capillary tubes after centrifugation in a hematocrit centri fuge (AB L. Ljungberg & Co Stockholm) From the recorded values of Hb and Hct, the MCHC was calculated.

The details of the methodology are consistent with the standard procedure used by Natvig et al. [38] in the comprehensive study of the hemoglobin values in Norway

Routinely the determinations of Hb and Hct were performed in blood samples col lected in heparinized centrifuge tubes after venepuncture of the antecubital vein. In the schoolchildren, however the determinations were performed in blood samples taken from a prewarmed fingertip, by pricking with lancets.

The blood samples from the students of the primary material (A) and the supple mentary material (B) were obtained in the afternoon. In the remainder of the material the blood sampling usually was performed earlier in the day

Serum iron TIBC and percent saturation of transferrin

The Teepol bathophenanthroline method modified by Askevold & Vellar [3] was used for the determination of serum iron. The TIBC was measured by the method described by Ramsay [41] although the final step was modified and adopted to the Teepol-bathopenanthroline procedure for the serum iron determination. From the measured values of serum iron and TIBC, the transferrin sat% was calculated (serum iron as a percentage of TIBC)

The total quantity of hemoglobin

The total quantity of hemoglobin (THb) was calculated from estimations of Hb concentration and blood volume. The blood volume was calculated from Hct and the plasma volume which was measured accord ing to the radio-iodine plasma protein method of Williams & Fine [57] Ten ml of an isotonic NaCl-solution, containing 1.2 % albumin labeled with ^{131}J and with an activi ty of 0.3 μCi per ml, was injected intra venously and the degree of dilution was taken as a measure of the plasma volume of the subject. After a period of 10 min, allowing a thorough mixing between the injected albumin-^{131}J and the plasma protein pool, a blood sample of 15 ml was withdrawn into a heparinized syringe. The blood was centrifuged and the isotopic measure-

RESULTS

A. CROSS-SECTIONAL ANALYSIS IN THE PRIMARY MATERIAL

Values for hematological indices and physical work capacity in students

The results of the determinations of hematological indices and maximal oxygen uptake at the six examinations are presented in Table V (male students) and Table VI (female students).

The mean values for Hb were lower than the optimal mean values suggested by Natvig & Vellar [39]. This tendency was particularly pronounced for the female students at the first examination. Many of the serum iron values were also low at least compared with the normal range used in Rikshospitalet [34]. The variations in the mean values for serum iron, TIBC and transferrin sat% however were noticeable from examination to examination in both male and female students.

The mean values of the maximal oxygen uptake (indirect method) at the start of the examination period were slightly higher (5—10 %) than previously found by Hermansen & Lange Andersen [29] in untrained Norwegian students of comparable age. However the mean values obtained at the second to the sixth examination were from 19 to 31 per cent higher than observed at the first examination.

In addition to the values for maximal oxygen uptake obtained with the indirect method and presented in Tables V and VI, a comparison of the direct and the indirect

Table V. Data obtained in the male students of the primary material at each of the six examinations (mean ± standard error). The maximal oxygen uptake is estimated by the indirect method

Examination	1st	2nd	3rd	4th	5th	6th
No. in group	47	44	42	41	45	44
Body height (cm)	179.5 ± 1.0	–	–	–	–	–
Body weight (kg)	74.50 ± 1.23	75.13 ± 1.24	75.12 ± 1.26	76.13 ± 1.19	75.61 ± 1.15	74.93 ± 1.22
Hematological indices						
Hb (g/100 ml)	15.10 ± 0.12	15.04 ± 0.12	15.08 ± 0.12	14.84 ± 0.11	15.70 ± 0.13	15.19 ± 0.15
Hct (%)	46.0 ± 0.4	44.4 ± 0.3	45.2 ± 0.3	44.6 ± 0.4	46.6 ± 0.4	46.1 ± 0.3
MCHC %	32.87 ± 0.16	33.87 ± 0.18	33.36 ± 0.21	33.31 ± 0.20	33.68 ± 0.16	32.97 ± 0.22
Serum iron (μg/100 ml)	114.0 ± 5.0	88.3 ± 3.9	102.5 ± 5.4	76.7 ± 7.0	(140.4 ± 7.8)*	104.0 ± 4.7
TIBC (μg/100 ml)	346.7 ± 7.1	343.5 ± 6.8	337.4 ± 6.6	325.6 ± 6.4	(414.2 ± 15.1)*	339.4 ± 7.1
Transferrin (sat %)	33.7 ± 1.9	26.3 ± 1.6	30.9 ± 2.0	24.3 ± 2.6	(36.4 ± 2.8)	30.8 ± 1.3
Maximal oxygen uptake						
(liter/min)	3.52 ± 0.08	4.13 ± 0.08	4.07 ± 0.10	4.18 ± 0.10	4.40 ± 0.11	[illegible]
(ml/kg min)	47.50 ± 1.11	55.33 ± 1.12	54.47 ± 1.10	54.88 ± 1.27	[illegible]	[illegible]

Estimation of serum iron and TIBC was performed in samples after storage in [illegible]

Table VI. Data obtained in the female students of the primary material at each of the six examinations (mean ± standard error). The maximal oxygen uptake is estimated by the indirect method

Examination	1st	2nd	3rd	4th	5th	6th
No. in group	50	49	48	44	47	46
Body height (cm)	167.8 ± 0.9	–	–	–	–	–
Body weight (kg)	59.44 ± 0.92	60.34 ± 0.93	60.31 ± 0.91	60.07 ± 0.96	60.83 ± 0.97	60.04 ± 0.93
Hematological indices						
Hb (g/100 ml)	12.91 ± 0.12	13.31 ± 0.11	13.53 ± 0.09	13.53 ± 0.1	14.15 ± 0.12	13.77 ± 0.11
Hct (%)	39.5 ± 0.3	40.9 ± 0.4	42.0 ± 0.3	42.0 ± 0.4	43.3 ± 0.4	42.7 ± 0.4
MCHC (%)	32.62 ± 0.12	32.57 ± 0.14	32.21 ± 0.11	32.21 ± 0.17	32.72 ± 0.17	32.22 ± 0.15
Serum iron (μg/100 ml)	88.2 ± 4.8	59.3 ± 2.4	75.7 ± 3.8	87.0 ± 4.4	(117.3 ± 4.7)	89.4 ± 4.7
TIBC (μg/100 ml)	351.9 ± 7.0	364.5 ± 7.1	368.4 ± 6.7	356.7 ± 7.9	(448.4 ± 12.4)	335.8 ± 5.9
Transferrin (sat. %)	25.7 ± 1.3	16.6 ± 0.8	21.1 ± 1.[illegible]	25.2 ± 1.6	(26.8 ± 1.2)*	27.1 ± 1.5
Maximal oxygen uptake						
(liter/min)	2.42 ± 0.06	3.32 ± 0.11	3.04 ± 0.08	2.78 ± 0.06	3.48 ± 0.10	3.20 ± 0.09
(ml/kg × min)	40.88 ± 0.94	54.93 ± 1.46	50.55 ± 1.22	46.28 ± 1.00	57.42 ± 1.45	53.54 ± 1.39

Estimation of serum iron and TIBC was performed in samples after storage in a freezer

measurement of maximal oxygen uptake was performed in 20 male and 21 female students at the first examination, and in 16 male and 15 female students at the sixth examination.

The mean maximal oxygen uptake, indirect method, in the 20 male students at the first examination was 4.0 liter/min compared with 4.4 liter/min in the same subjects when using the direct method. The corresponding figures for 16 men at the sixth examination were 4.7 and 4.9 liter/min. In the 21 female students, the mean maximal oxygen uptake, indirect method, at the first examination was 2.4 liter/min compared with 2.7 liter/min for the direct method. In 15 women at the sixth examination, however the indirect method gave a slightly higher value (3.4 liter/min) than the direct method (3.1 liter/min).

Thus, the indirect method gave mean values which in the various subgroups represented 89—110 per cent of the corresponding direct estimates.

Values for hematological indices and physical work capacity in schoolchildren

The results of the determinations of hematological indices and maximal oxygen uptake in the schoolchildren who were examined at both the first and the final examination, are presented in Table VII (schoolboys) and Table VIII (schoolgirls) and given for each age group. The time interval between the two examinations was approximately six months. Corresponding data for the total number of schoolchildren examined at the final examination are given in Table IX (schoolboys) and Table X (schoolgirls).

The mean values for Hb were fairly well in accordance with the proposed normal values of Natvig et al. [40] for schoolchildren of comparable age. In some of the groups, however the mean values were below the previously proposed means.

The values for maximal oxygen uptake (Table VII—X) were on average slightly

Table VII. Data obtained in the 39 schoolboys (primary material) who were examined at both the first and the final examination (mean ± standard error)

Age (yrs)	12		13		14	
Examination	First	Final	First	Final	First	Final
No. in group	13	13	22	22	4	4
Body height (cm)	148.5 ± 2.0	151.1 ± 2.4	154.5 ± 1.27	156.7 ± 1.2	149.5 ± 1.9	152.0 ± 2.9
Body weight (kg)	37.75 ± 1.55	40.28 ± 1.91	43.51 ± 0.90	46.04 ± 1.00	39.96 ± 2.89	41.58 ± 3.23
Hematological indices						
Hb (g/100 ml)	12.93 ± 0.20	13.22 ± 0.23	12.97 ± 0.12	13.17 ± 0.18	13.05 ± 0.15	13.48 ± 0.25
Hct (%)	40.0 ± 0.6	40.7 ± 0.7	39.3 ± 0.4	39.5 ± 0.4	39.5 ± 0.5	40.5 ± 1.3
MCHC (%)	32.61 ± 0.35	32.48 ± 0.32	32.99 ± 0.27	33.27 ± 0.26	33.00 ± 0.66	33.35 ± 0.51
Maximal oxygen uptake						
Indirect method						
(liter/min)	1.88 ± 0.08	1.94 ± 0.12	2.19 ± 0.08	2.27 ± 0.10	2.35 ± 0.10	2.45 ± 0.20
(ml/kg min)	51.35 ± 2.17	48.24 ± 2.07	50.46 ± 1.90	49.51 ± 2.07	59.50 ± 4.13	59.03 ± 2.84
Direct method						
(liter/min)	2.38 ± 0.09	2.63 ± 0.11	2.63 ± 0.06	2.95 ± 0.08	2.59 ± 0.13	2.80 ± 0.29
(ml/kg min)	63.00 ± 1.53	65.63 ± 1.12	60.57 ± 0.89	64.17 ± 1.16	65.20 ± 2.92	64.90 ± 0.55

Table VIII. Data obtained in the 33 schoolgirls (primary material) who were examined at both the first and the final examination (mean ± standard error)

Age (yrs)	11		12		13	
Examination	First	Final	First	Final	First	Final
No. in group	1	1	16	16	16	16
Body height (cm)	145	147	151.2 ± 1.7	152.8 ± 1.6	156.4 ± 1.1	157.9 ± 1.2
Body weight (kg)	43.0	46.0	41.83 ± 1.43	44.28 ± 1.49	47.71 ± 1.84	50.31 ± 1.81
Hematological indices						
Hb (g/100 ml)	13.3	13.5	12.84 ± 0.24	12.94 ± 0.20	13.28 ± 0.13	13.25 ± 0.13
Hct (%)	39	41	39.4 ± 0.5	40.3 ± 0.5	40.7 ± 0.3	39.7 ± 0.3
MCHC (%)	34.0	32.9	32.36 ± 0.28	32.13 ± 0.29	32.61 ± 0.29	33.35 ± 0.28
Maximal oxygen uptake						
Indirect method						
(liter/min)		1.4	1.69 ± 0.08	1.79 ± 0.05	1.93 ± 0.05	2.07 ± 0.06
(ml/kg min)		30.4	43.73 ± 2.76	40.93 ± 1.47	40.94 ± 1.43	41.57 ± 2.07
Direct method						
(liter/min)	2.0	2.2	2.31 ± 0.06	2.46 ± 0.08	2.47 ± 0.07	2.68 ± 0.06
(ml/kg min)	46.7	48.7	55.74 ± 1.15	56.05 ± 1.54	52.17 ± 1.17	53.75 ± 1.46

Table IX. Data obtained in all 95 schoolboys (primary material) at the final examination (mean ± standard error)

Age (yrs)	10	11	12	13	14	15	16	Total
No in group	9	8	23	31	10	10	4	95
Body height (cm)	138.9 ± 3.1	146.4 ± 1.6	148.7 ± 1.6	156.0 ± 1.2	162.0 ± 3.6	165.5 ± 2.5	172.8 ± 4.7	154.1 ± 1.2
Body weight (kg)	33.10 ± 2.66	37.95 ± 1.54	40.10 ± 1.35	47.02 ± 1.05	50.92 ± 3.16	55.31 ± 2.42	61.85 ± 3.45	45.15 ± 1.01
Hematological indices								
Hb (g/100 ml)	12.82 ± 0.30	13.29 ± 0.27	13.14 ± 0.22	13.21 ± 0.13	13.93 ± 0.39	14.01 ± 0.36	14.55 ± 0.44	13.38 ± 0.10
Hct (%)	40.1 ± 0.6	40.6 ± 0.7	40.5 ± 0.5	40.1 ± 0.4	42.8 ± 1.0	44.1 ± 0.7	45.0 ± 1.7	41.2 ± 0.3
MCHC (%)	31.91 ± 0.45	32.65 ± 0.29	32.43 ± 0.26	32.27 ± 0.68	32.56 ± 0.52	31.78 ± 0.58	32.38 ± 0.5[illegible]	3[illegible].29 ± 0.24
Maximal oxygen uptake								
Indirect method								
(liter/min)	1.38 ± 0.06	1.55 ± 0.10	1.85 ± 0.08	2.24 ± 0.08	2.47 ± 0.12	2.36 ± 0.17	2.78 ± 0.11	2.07 ± 0.05
(ml/kg min)	44.10 ± 3.97	41.19 ± 2.97	46.51 ± 1.45	48.03 ± 1.69	49.74 ± 3.08	42.36 ± 2.93	45.15 ± 2.[illegible]6	46.17 ± 0.92
Direct method								
(liter/min)	1.91 ± 0.10	2.29 ± 0.11	2.56 ± 0.08	3.01 ± 0.07	3.37 ± 0.23	3.37 ± 0.16	3.73 ± 0.33	2.83 ± 0.07
(ml/kg min)	56.07 ± 2.95	60.41 ± 1.59	63.90 ± 0.84	64.25 ± 1.00	64.00 ± 1.32	61.11 ± 1.04	59.98 ± [illegible].73	62.50 ± 0.58

Table X. Data obtained in all 88 schoolgirls (primary material) at the final examination (mean ± standard error)

Age (yrs)	10	11	12	13	14	15	16	Total
No in group	5	11	30	23	7	5		88
Body height (cm)	137.2 ± 0.3	148.0 ± 2.5	151.8 ± 1.5	157.3 ± 0.9	156.3 ± 0.7	157.0 ± 2.8	162.1 ± 3.5	153.4 ± 0.9
Body weight (kg)	33.18 ± 1.96	38.52 ± 1.94	43.27 ± 1.50	50.76 ± 1.44	53.10 ± 2.47	48.36 ± 1.80	53.34 ± 3.00	43.96 ± 0.9[illegible]
Hematological indices								
Hb (g/100 ml)	13.24 ± 0.21	12.98 ± 0.16	12.72 ± 0.13	12.96 ± 0.15	12.86 ± 0.18	12.70 ± 0.30	12.31 ± 0.49	1[illegible].82 ± 0.79
Hct (%)	40.2 ± 0.7	40.5 ± 0.5	39.8 ± 0.3	39.7 ± 0.2	41.0 ± 0.8	41.0 ± 1.0	39.1 ± 1.1	40.0 ± 0.2
MCHC (%)	32.94 ± 0.47	31.96 ± 0.23	31.99 ± 0.19	32.66 ± 0.32	31.34 ± 0.27	30.96 ± 0.67	31.34 ± 0.48	32.03 ± 0.13
Maximal oxygen uptake								
Indirect method								
(liter/min)	1.50 ± 0.58	1.60 ± 0.12	1.76 ± 0.04	2.05 ± 0.05	2.03 ± 0.13	1.84 ± 0.16	2.21 ± 0.25	1.88 ± 0.40
(ml/kg min)	44.00 ± 4.09	39.64 ± 2.29	41.45 ± 1.34	40.79 ± 1.69	38.10 ± 1.33	38.12 ± 2.98	42.30 ± 5.67	40.77 ± 0.88
Direct method								
(liter/min)	1.71 ± 0.10	2.06 ± 0.10	2.35 ± 0.07	2.61 ± 0.05	2.52 ± 0.11	2.44 ± 0.14	2.53 ± 0.14	2.37 ± 0.04
(ml/kg min)	51.46 ± 0.64	53.60 ± 1.26	54.75 ± 1.10	51.87 ± 1.30	47.70 ± 1.99	50.36 ± 1.42	47.66 ± 0.54	52.27 ± 0.61

higher (5—10 %) than the values given by Astrand [5] for schoolchildren in Stock holm of comparable age.

It should be noted that the mean values for maximal oxygen uptake, indirect method, were approximately 20—25 per cent lower than the corresponding values determined by the direct method.

The well known increase in maximal oxygen uptake with age was also demonstrated in the present investigation (Tables VII—X)

Correlations between hematological indices and physical work capacity

Total primary material The results of the correlation analysis concerning the relationship between corresponding values for the various hematological indices (Hb Hct, MCHC, serum iron, TIBC, transferrin sat% and THb) and the maximal oxygen uptake in the total primary material at the first and the sixth examination, are presented in Tables XI—XIV

Correlation coefficients have been calcu lated both from maximal oxygen uptake data in *liter/min* (indirect method Table XI and direct method Table XII) and in *ml/kg × min* (indirect method Table XIII, and direct method Table XIV)

The results obtained in each of the examinations have been analyzed separately Thus, each of the correlation coefficients is referring to only one set of values in each individual. It should be noted that the results obtained at the first examination both in students and in schoolchildren are pooled and designated *1st examination* although the investigations in the two groups were undertaken at different calendar times. In the same way the results obtained at the sixth examination in the students and at the final examination in the schoolchildren, are pooled and designat ed *6th examination* (i.e. final examination)

T ble XI Correlations ( ) between hematological indices and maximal oxygen uptake in liter/min (*indirect method*) in the total primary material at the first and the sixth examination (serum iron, TIBC transferrin (sat %) and THb were only determined in students)

Hematological Indices	Maximal oxygen uptake (indirect method) in liter/min — Examination: 1st (Students & schoolchildren)	6th (Students & schoolchildren)
Hb	+0.58	+0.59
	p < 0.001 (= 157)	p < 0.001 (= 256)
Hct	+0.58	+0.61
	p 0.001 (= 156)	p 0.001 (= 256)
MCHC	+0.09	+0.13
	Not sign. (n = 155)	0.01 p < 0.05 (n = 256)
Serum iron	+0.34	+0.14
	p 0.001 (= 96)	Not sign. (= 88)
TIBC	−0.08	−0.06
	Not sign. (= 89)	Not sign. (n = 88)
Transferrin (sat %)	−0.33	+0.13
	0.001 p 0.01 (= 89)	Not sign. (= 88)
THb	+0.76	+0.69
	p 0.001 (= 20)	p < 0.001 (= 24)

T ble XII. Correlations (r) between hematological indices and maximal oxygen uptake in liter/min (*direct method*) in the total primary material at the first and the sixth examination (serum iron, TIBC, transferrin (sat %) and THb were only estimated in students)

Hematological Indices	Maximal oxygen uptake (direct method) in liter/min — Examination: 1st (Students & schoolchildren)	6th (Students & schoolchildren)
Hb	+0.67	+0.59
	p < 0.001 (= 109)	p < 0.001 (n = 208)
Hct	+0.69	+0.61
	p < 0.001 (n = 108)	p < 0.001 (n = 205)
MCHC	+0.06	+0.08
	Not sign. (= 106)	Not sign. (n = 208)
Serum iron	+0.21	+0.12
	Not sign. (= 41)	Not sign. (n = 31)
TIBC	−0.11	−0.11
	Not sign. (= 40)	Not sign. (n = 31)
Transferrin (sat %)	+0.20	+0.13
	Not sign. (n = 40)	Not sign. (= 31)
THb	+0.85	+0.70
	p < 0.001 (n = 20)	p < 0.001 (= 22)

Table XIII. Correlations (r) between hematological indices and maximal oxygen uptake in ml/kg × min (*indirect method*) in the total primary material at the first and the sixth examination (serum iron, TIBC, transferrin (sat %) and THb were only determined in students)

Hematological indices	Maximal oxygen uptake (indirect method) in ml/kg × min. Examination: 1st (Students & schoolchildren)	6th (Students & schoolchildren)
Hb	−0.04	−0.35
	Not sign. (n = 157)	p 0.001 (n = 256)
Hct	−0.03	−0.32
	Not sign. (n = 156)	p < 0.001 (n = 256)
MCHC	−0.03	−0.13
	Not sign. (n = 155)	0.01 p < 0.05 (n = 256)
Serum	−0.[illegible]8	−0.01
iron	0.001 < p < 0.01 (n = 96)	Not sign. (n = 88)
TIBC	−0.17	−0.05
	Not sign. (n = 89)	Not sign. (n = 88)
Transferrin	−0.31	0.00
(sat %)	0.001 < p 0.01 (= 89)	Not sign. (n = 88)
THb	−0.35	−0.03
	Not sign. (n = 20)	Not sign. (n = 4)

Table XIV. Correlations (r) between hematological indices and maximal oxygen uptake in ml/kg × min (*direct method*) in the total primary material at the first and the sixth examination (serum iron, TIBC, transferrin (sat %) and THb were only estimated in students)

Hematological indices	Maximal oxygen uptake (direct method) in ml/kg × min. Examination: 1st (Students & schoolchildren)	6th (Students & schoolchildren)
Hb	−0.07	−0.[illegible]4
	Not sign. (= 90)	p < 0.001 (n = 208)
Hct	−0.10	−0.17
	Not sign. (n = 109)	0.01 < p < 0.05 (n = 208)
MCHC	−0.03	+0.09
	Not sign. (= 106)	Not sign. (= 208)
Serum	−0.25	−0.07
iron	Not sign. (= 41)	Not sign. (n = 31)
TIBC	−0.13	−0.[illegible]8
	Not sign. (= 40)	Not sign. (n = 31)
Transferrin	0.25	+0.13
(sat %)	Not sign. (n = 40)	Not sign. (n = 31)
THb	−0.54	+0.23
	0.01 < p < 0.05 (n = 20)	Not sign. (n = 22)

There was a statistically significant, positive correlation between the Hb-concentration and the maximal oxygen uptake (liter/min) when measured indirectly (Table XI) as well as directly (Table XII). The level of significance was high ($p<0.001$). With regard to the total quantity of hemoglobin (THb) a similar result was found. Although the number of observations for this hematological parameter was rather small, the correlation coefficients reached a high level of significance ($p<0.001$). A similar statistically significant positive association was also found between the Hct and the maximal oxygen uptake.

When the data for maximal oxygen uptake were given as *ml/kg × min* (Tables XIII and XIV) no definite statistical relationship was found between Hb and Hct and maximal oxygen uptake at the first examination. At the sixth examination, however a statistical relationship was found. It should be noted, however that the number of schoolchildren in the composite material had been markedly increased.

When maximal oxygen uptake was measured directly (Tables XII and XIV) there was no relationship between MCHC and the three parameters related to serum iron (serum iron, TIBC and percent saturation of transferrin) and maximal oxygen uptake, expressed as liter/min or ml/kg × min.

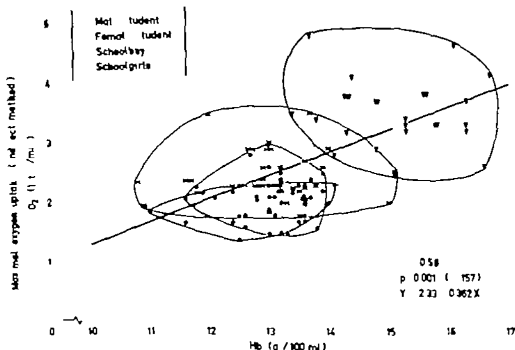
When indirect measurements were used (Tables XI and XIII) the number in each group was greater and six of 16 correlation coefficients were now positive and statistically significant. The coefficients for MCHC, however were only significant at the sixth examination when many schoolchildren participated, but not at the first examination. The coefficients for serum iron and transferrin sat% were on the contrary only significant at the first examination. At this time 16 of the students were iron-deficient as revealed by sub-normal Hb-

Mal student
F male tud ts
Sch olb ys
Schoolg l

Ma mal yg uptak (d t method) O2 (lit /min)

0,87
p 0,001 (109)
Y 3,88 0,503X

Hb (g / 100 ml)



Figs 3 a (upper panel) and 3 b (lower panel) Corresponding values for maximal oxygen uptake in liter min (direct method in the upper panel and indirect method in the lower panel) and hemoglobin concentration in the primary material at the first examination. The values in each of the four subgroups are encircled. There were no significant associations between th two parameters within these subgroups, with the exception of schoolgirls, using the direct method ($= + 0.55$, $0.001 < p < 0.01$, $n = 31$)

values. These correlation coefficients were significant when maximal oxygen uptake was expressed as liter/min as well as ml/kg × min.

Subgroups of the primary material In the total primary material there was a close positive correlation between the Hb-concentration and the maximal oxygen uptake (liter/min) In order to make a further assessment of this relationship, the corresponding correlation coefficients for the various subgroups (male and female students, schoolboys and schoolgirls) have also been calculated. With a few exceptions, none of the subgroup correlation coefficients were statistically significant. A similar lack of relationship was found when the corresponding subgroup correlation coefficients for Hct and MCHC were examined.

The general finding is illustrated in Fig.3 a (direct method) and Fig 3 b (indirect method) Corresponding values for maximal oxygen uptake in liter/min and the Hb-concentration are given for the total primary material at the first examination. It is evident that although the scatter of the individual values around the calculated regression lines is considerable, the correlation between the two parameters is positive and highly significant ($r = +0.67$ and $+0.68$ respectively) However within the four subgroups (male and female students, schoolboys and schoolgirls) which are encircled on the figures, there is no definite correlation between the hematological parameter and the physical work capacity The only exception was a positive and statistically significant correlation ($r = +0.55$ $0.001 < p < 0.01$, $n = 31$) within the schoolgirl subgroup, but only when the direct method was used. A similar correlation could not be demonstrated using the indirect method.

Although there is a great deal of overlapping in the individual values of the various subgroups, there is a *tendency* however to a concomitant increase in both maximal oxygen uptake and Hb-concentration from subgroup to subgroup in the following order schoolgirls, schoolboys, female students, male students.

A similar covariation between changes in Hb-concentration and maximal oxygen uptake (liter/min) may also explain the finding at the sixth examination of a positive and statistically significant correlation coefficient in the schoolboy subgroup. At this examination the age-span of the 93 schoolbooys was wide (10—16 yrs.) and consequently some of the individuals were only small boys who were compared with others who were nearly mature.

There was no definite relationship between the values for serum iron and maximal oxygen uptake in the students (both sexes) The same lack of relationship was found when the correlation coefficients

Table XV Correlations ( ) between the total amount of hemoglobin (THb) and the maximal oxygen uptake in liter/min (*indirect and direct methods*) in the various groups of students of the primary material at the first and the sixth examination

	Maximal oxygen uptake (liter/min)			
	Indirect method Examination		Direct method Examination	
Material	1st	6th	1st	6th
Students (both sexes)	0.76 p 0.001 (n 20)	+0.69 p 0.001 (n = 24)	+0.85 p < 0.001 (n = 20)	+0.70 p 0.001 (n = 22)
Males	0.34 Not sign (n 10)	+0.44 Not sign. (n = 13)	+0.49 Not sign. (= 10)	+0.51 Not sign. (= 13)
Females	0.49 Not sign (10)	0.28 Not sign. (n = 11)	+0.66 0.01 < p 0.05 (n = 10)	−0.28 Not sign. (n = 9)

Table XVI. Correlations (r) between the total amount of hemoglobin (THb) and the maximal oxygen uptake in ml/kg × min (*indirect* and *direct methods*) in the arious groups of students of the primary material at the first and the sixth examination

Material	Maximal oxygen uptake (ml/kg × min)			
	Indirect method Examination		Direct method Examination	
	1st	6th	1st	6th
Students	+0.35	−0.03	−0.54	−0.23
(both sexes)	Not sign. (= 20)	Not sign. (n = 24)	$0.01 < p < 0.05$ (= 20)	Not sign. (n = 22)
Males	−0.15	−0.43	−0.16	−0.50
	Not sign. (= 10)	Not sign. (= 13)	Not sign. (= 10)	Not sign. (= 13)
Females	−0.09	−0.23	−0.12	+0.15
	Not sign. (n = 10)	Not sign. (= 11)	Not sign. (= 10)	Not sign. (= 9)

were calculated separately for each sex. Similar results were obtained for TIBC and percent saturation of transferrin.

Table XV shows that the close correlation which was found in the students (both sexes) between the total amount of hemoglobin (THb) and the maximal oxygen uptake (liter/min) could not be confirmed when each sex was examined separately. The same lack of relationship was found when the correlation coefficients were based on maximal oxygen uptake in ml/kg × min (Table XVI). The number in each group, however, was small.

B. LONGITUDINAL ANALYSIS IN THE PRIMARY STUDENT MATERIAL

The results of the six examinations have also been compared longitudinally (Tables V and VI).

Although there were some fluctuations in the values from examination to examination, there was a pronounced increase in maximal oxygen uptake during the examination period in both male and female students. The mean increase in maximal oxygen uptake (indirect method) from the first to the sixth examination was about 12 ml/kg × min in both sexes (25—31 per cent above the initial values). In the female students, a noticeable increase was also found in the Hb-concentration (7 per cent above the initial value) in the same period, but not in the male students.

In both sexes there were also substantial fluctuations in the serum iron levels, without any special tendency to increase or decrease. It should be noted, however, that the values for both Hb and serum iron in both sexes reached the highest levels at the fifth examination. This was also found for maximal oxygen uptake, but only in female students.

C. *EXPERIMENTS WITH IRON AND* PLACEBO

Subgroups of the primary student material

As shown in Tables XVII (male students) and XVIII (female students) the subgroups within each sex were comparable both with regard to maximal oxygen uptake and hematological indices (with the exception of the subnormal group) at the start of the experimental period.

The assessment of the actual consumption of tablets (iron or placebo) at the end of the experiment revealed that the students had scrupulously followed the instructions to take one tablet a day during the experimental period (40 weeks). The average consumption per individual was about 270 tablets, and thus in good agreement with the theoretical consumption according to the plan of the experiment (approximately 280 tablets).

The values for maximal oxygen uptake and Hb-concentration for the various subgroups of the male and female students

during the course of the experimental period are given in Figs. 4 and 5. The same increase in maximal oxygen uptake as shown in all male students(Table V) and all female students (Table VI) of the primary material, was also found in the different subgroups. Although there were some fluctuations in the values, no system

Table XVII. Data obtained in the various groups (normal or sub-normal Hb-concentration, iron or placebo treatment) of the male students at the start of the experimental period (1st examination). The maximal oxygen uptake is estimated by the indirect method

	Male students with normal Hb (> 14.0 g/100 ml)		Male students with sub-normal Hb (< 14.0 g/100ml)	
	Iron	Placebo	Iron	Total
No. in group	21	1	5	47
Body height (cm)	181.4 ± 1.5	177.7 ± 1.3	180.2 ± 3.7	179.5 ± 1.0
Body weight (kg)	76.26 ± 1.66	71.70 ± 1.71	78.84 ± 5.18	74.50 ± 1.23
Hematological indices				
Hb (g/100 ml)	15.30 ± 0.16	15.23 ± 0.16	13.68 ± 0.86	15.10 ± 0.12
Hct (%)	46.6 ± 0.5	46.0 ± 0.6	43.0 ± 0.9	46.0 ± 0.4
MCHC (%)	32.85 ± 0.20	33.1 ± 0.25	31.86 ± 0.33	32.87 ± 0.16
Serum iron (µg 100 ml)	118.8 ± 6.8	112.9 ± 8.8	98.6 ± 7.7	114.0 ± 5.0
TIBC (µg 100 ml)	352.5 ± 11.[illegible]	332.6 ± 8.4	381.8 ± 28.1	346.7 ± 7.1
Transferrin (sat %)	34.3 ± 2.1	34.9 ± 3.5	26.1 ± 2.3	33.7 ± 1.9
Maximal oxygen uptake				
(liter/min)	3.55 ± 0.10	3.43 ± 0.14	3.76 ± 0.25	3.52 ± 0.08
(ml/kg min)	46.67 ± 1.31	47.93 ± 1.62	49.20 ± 6.42	47.50 ± 1.11

Table XVIII. Data obtained in the various groups (normal or sub-normal Hb concentration, iron or placebo treatment) of the female students at the start of the experimental period (1st examination). The maximal oxygen uptake is estimated by the indirect method

	Female students with normal Hb (> 12.5 g 100 ml)		Female students with sub-normal Hb (< 1[illegible].5 g/100 ml)	
	Iron	Placebo	Iron	Total
No. [illegible] group	19	20	11	50
Body [illegible]ht (cm)	168.1 ± 1.3	165.2 ± 1.3	171.6 ± 2.2	167.8 ± 0.9
Body [illegible] (kg)	61.05 ± 1.21	57.28 ± 1.58	60.41 ± 2.08	59.44 ± 0.9[illegible]
Hematological indices				
Hb (g [illegible]0 ml)	13.22 ± 0.13	13.23 ± 0.14	11.79 ± 0.17	1[illegible].91 ± 0.12
Hct	40.2 ± 0.5	40.5 ± 0.4	36.7 ± 0.4	39.5 ± 0.3
MCHC	32.79 ± 0.16	32.74 ± 0.19	32.09 ± 0.27	32.6[illegible] ± 0.12
Serum [illegible] µg 1[illegible] ml)	92.5 ± 6.8	97.7 ± 8.1	63.5 ± 8.0	88.2 ± 4.8
TIBC µg 100 m[illegible]	360.5 ± 11.4	348.9 ± 10.5	341.3 ± 16.7	351.9 ± 7.0
Transfer[illegible] (sat [illegible]	26.2 ± 2.1	28.6 ± 2.[illegible]	19.[illegible] ± [illegible].1	25.7 ± 1.3
Maximal [illegible] uptake				
(liter/min)	[illegible]43 ± 0.11	2.43 ± 0.10	2.40 ± 0.1	2.42 ± 0.06
(ml/kg [illegible])	39.86 ± 1.76	4[illegible].41 ± 1.28	39.99 ± 1.87	40.88 ± 0.94

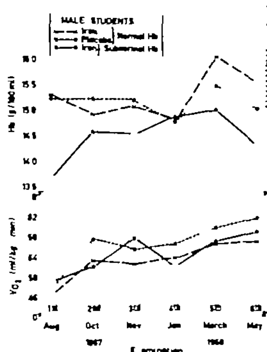


Fig. 4 The mean values for hemoglobin concentration and maximal oxygen uptake (indirect method) in the three subgroups of the male students (primary material) during the course of the experimental period (first to sixth examination)

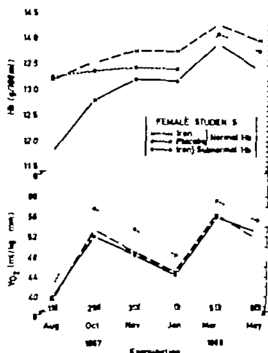


Fig 5 The mean values for hemoglobin concentration and maximal oxygen uptake (indirect method) in the three subgroups of the female students (primary material) during the course of the experimental period (first to sixth examination)

atic difference was found between the groups throughout the experimental period.

The Hb-concentration increased in all *female* subgroups, *although not to the same* extent in the normal groups as found in the sub-normal group. A similar increase, however was not found in the male subgroups.

It should be noted that there was no noticeable *difference* between the Hb-concentrations of the normal placebo and the normal iron groups during the course of the experimental period (nine months) neither in male nor in female subjects. Furthermore, Figs. 4 and 5 demonstrate that maximal oxygen uptake may increase and Hb-concentration decrease, or vice versa, during the same experimental interval, i.e. from one examination to another

A similar lack of relationship between changes in physical work capacity and variations in the other hematological indices (Hct, MCHC, serum iron, TIBC and percent saturation of transferrin) was also observed.

Supplementary material of female students

In the primary material of students a pronounced increase in physical work capacity was *found during the initial* eight weeks between the first and the second examination. This increase is further elucidated in Fig 6, giving the weekly changes in maximal oxygen uptake in the various subgroups of the supplementary material of female students (low normal Hb Iron or placebo/iron treatment subnormal Hb iron or placebo/iron treatment)

As shown the total increase in maximal oxygen uptake (direct method) during the seven weeks *period is of the same order of*

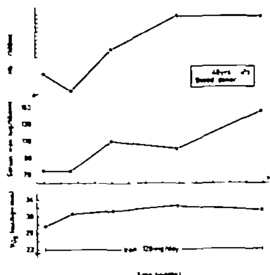


Fig. 7 The values for hemoglobin concentration, serum iron and maximal oxygen uptake (direct method) in a mal blood donor (T H.) with iron-deficiency anemia, during the course of iron therapy (13 weeks)

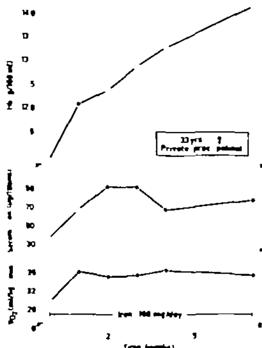


Fig. 8. The values for hemoglobin concentration, serum iron and maximal oxygen uptake (direct method) in a female patient (T H.) with iron-deficiency anemia, during the course of iron therapy (seven weeks)

There was also an increase although irregular in serum iron, but no further increase in maximal oxygen uptake.

Subject R.L. (female 35 yrs.) blood donor The maximal oxygen uptake (direct method) at the start of the experiment was 35.3 ml/kg × min (Fig 9) and the hematological status was consistant with a moderate degree of iron-deficiency anemia (Hb 11.0 g/100 ml, Hct 35 % serum iron 41 μg/100 ml, TIBC 398 μg/100 ml and transferrin sat% 10.3) Iron treatment (120 mg per day) was started.

During the first four weeks of the experiment, the Hb increased from 11.0 to 11.9 g/100 ml. A corresponding increase was recorded neither in serum iron, nor in maximal oxygen uptake.

During the next four weeks all three parameters were constant, whereas during the last 12 weeks both Hb and maximal oxygen uptake showed a small but consistant increase. The changes in the serum iron level were more irregular

Subject S.H (female 18 yrs.) blood donor The maximal oxygen uptake (direct method) at the start of the experiment was 37.0 ml/kg × min (Fig. 10) and the hematological status was compatible with a moderate degree of iron-deficiency anemia (Hb 11.4 g/100 ml, Hct 34 % serum iron 44 μg/100 ml, TIBC 338 μg/100 ml and transferrin sat% 13.0) In this case, placebo treatment was initiated, but replaced by iron treatment after four weeks. During the four weeks of placebo treatment, no change was found in the Hb-concentration, whereas the serum iron level increased considerably A small, although sustained increase in maximal oxygen uptake was also observed.

When iron therapy replaced placebo treatment the Hb increased, and reached a level of approximately 12 g/100 ml which was kept constant throughout the experiment. A similar tendency however was neither observed in serum iron, nor in maximal oxygen uptake. In fact, the maximal oxygen uptake had the same value at the 4th and the 26th week.

Fig. 9 The values for hemoglobin concentration, serum iron and maximal oxygen uptake (direct method) in a female blood donor (R. L.) with iron-deficiency anemia, during the course of iron therapy (20 weeks)

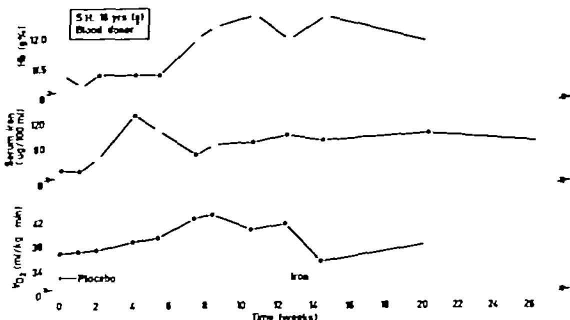


Fig 10 The values for hemoglobin concentration, serum iron and maximal oxygen uptak (direct method) in a female blood donor (S. H.) with iron-deficiency anemia during th course of treatment with placebo, later replaced by iron (26 weeks

DISCUSSION

Maximal oxygen uptake has widely been used as an index of physical performance [4, 6, 35 36, 47] as it gives valuable infor mation about both the functional capacity of the oxygen transport system (*hemoglobin*) and the maximal work power of the subjects. In the present investigation, maximal oxygen uptake (direct method) was measured during uphill (3) running at a speed which exhausted the subjects within 4—6 min. This particular procedure was chosen because the maximal rate of oxygen transport is affected by several factors, such as intensity and duration of the maximal work [9] the type of work [10] and the mass of muscles involved in the work [28, 31]

In order to ensure that the maximal oxygen uptake really was achieved, the levelling-off criterion was used [31] Furthermore, the blood lactate concentra tion and the heart rate response were also applied as additional criteria [5] However in some of the schoolchildren and in the clinical cases of anemia it was for practical reasons impossible to get more than one determination of the maximal oxygen uptake. Thus, the procedure in these experiments might give rise to some concern as to whether or not the values for maximal oxygen uptak really were maximal. How ever the speed and duration of the treadmill test was chosen according to the observations of a previous estimation taken six months before the start of the present study in the schoolchildren [30]

In the anemic cases the results of the previous test were used to determine the speed and duration of the next experiment. Additional information as to the choice of speed and duration was taken from the predicted value for maximal oxygen uptake (indirect method) as usually all subjects were tested on the bicycle ergometer before the maximal run was performed. This procedure provides a quick and reproducible method for the measurement of the individual s maximal oxygen uptake [47] Thus, there is good reasons to assume that the results were maximal, or near maximal, also in the experiments where the conventional levelling-off criterion was not used.

If the indirect method for the calculation of the maximal oxygen uptake is used as the only method, the methodological error is considerable [7] In the present study the indirect method gave mean values which in the various subgroups of the student material, represented 89—110 per cent of the corresponding direct estimates. However in the schoolchildren the discrepancy was somewhat more pronounced as the indirect method gave mean values which in the various age groups, represent ed 65—91 per cent of the corresponding direct estimates. It should be noted that the indirect method is a prediction of the maximal oxygen uptake from submaximal heart rate and work load with an extra polation to a maximal heart rate of 195 beats/min. Consequently in subjects with a maximal heart rate above 195 beats/min, the predicted values for maximal oxygen uptake (indirect method) will be lower than those obtained by the direct method.

In the present study the average maxi mal heart rates were 205 beats/min for the boys, and 207 beats/min for the girls. These maximal heart rates would at least partly explain the differences in the values obtained with the indirect and the direct methods.

The mean values for maximal oxygen uptake in the primary material of the present investigation were somewhat higher than previously found in Scandinvian schoolchildren and adults [4, 5, 29] With

regard to the students of the present study this difference may be explained by the fact that they were a highly selected group. A similar explanation may be relevant to the schoolchildren as they were sampled from two country schools in Finnmark. In comparison the Swedish schoolchildren examined by Astrand [5] lived in the City of Stockholm.

During the course of the nine months experimental period, the students of the present investigation (primary material) were submitted to a relatively high degree of training in some periods and to relatively little physical activity in other periods (theoretical sessions) This variation in physical activity may possibly explain the pronounced variations in the values for maximal oxygen uptake (indirect method) from one examination to another Although the indirect method does not provide the same degree of accuracy as the direct method, it gives valuable information about longitudinal changes as each individual serves as his own control. For instance, the incrase in maximal oxygen uptake (indirect method) which was found in the primary student material from the first to the second examination (8 weeks' interval) was confirmed in the supplementary material of female students when using the direct method. Thus, it seems unlikely that methodological errors in the indirect method may account for more than a fraction of the observed total variations in maximal oxygen uptake during the nine months experimental period.

With regard to the correlation analysis performed, it should be noted that the values for the various hematological indices covered a wide range. In the Hb values, for instance, the total range was from about 11 to about 17 g/100 ml. A similar picture was found for maximal oxygen uptake values.

A statistically significant positive correlation was found between the *hemoglobin concentration* and *maximal oxygen uptake (liter/min)* in the total primary material of the present investigation (schoolgirls, schoolboys, female students and male students) It should be noted that although there were some differences between measured (direct method) and predicted (indirect method) values for maximal oxygen uptake, the correlations in relation to hemoglobin concentration were almost identical. This observation with regard to the total primary material is in good agreement with earlier findings by Cullumbine [19] in a material of male and female subjects, aged 10 to 20 years. He found that the hemoglobin concentration (total range 8.9 — 19.7 g/100 ml) was directly associated with the speed of movement, strength and the ability to sustain prolonged muscular effort.

However the association between hemoglobin concentration and maximal oxygen uptake, expressed as liter/min, which was found in the present study with all subgroups combined, could not be confirmed when the correlation analysis was performed for each of the subgroups. The statistically significant correlation which was found in the total primary material, was apparently due to a concomitant increase (co-variation) in both hemoglobin concentration and maximal oxygen uptake (liter/min) from one subgroup to another in the following order schoolgirls, school boys, female students and male students.

This explanation is confirmed, at least partly by the results of the correlation analysis which was performed after elimination in the influence of the body weight, i.e. maximal oxygen uptake expressed as ml/kg × min. With such a correction, no reproducible significant association was found between the hemoglobin concentration and maximal oxygen uptake. At the sixth examination, however a statistical relationship was found. It should be noted that the number of schoolchildren in the composite material had been markedly increased at this examination.

Furthermore, the longitudinal comparisons of the results obtained at each of the six examinations in the students, revealed no relationship between the variations in hemoglobin concentration and the changes

in maximal oxygen uptake during the nine months' examination period. The maximal oxygen uptake increased considerably in both sexes, but the hemoglobin concentration increased noticeably only in the female students. With regard to the experiment with iron and placebo is should also be emphasized that the increase during the nine months' period in maximal oxygen uptake was of the same order of magnitude in all subgroups of the primary student material and also uninfluenced by the initial hemoglobin level. Furthermore, the fluctuations in maximal oxygen uptake and hemoglobin concentration during the same experimental interval, i.e. from one examination to another were often reciprocal. The same lack of association between hemoglobin concentration and maximal oxygen uptake was also observed in the supplementary material of female students with low normal/sub-normal hemoglobin concentration at the start of the iron/placebo experiment, as well as in the few selected cases of moderate or mild iron deficiency anemia.

Thus, cross-sectional as well as longitudinal analysis performed in the present study have revealed no obvious relationship between hemoglobin concentration (above 11 g/100 ml) and maximal oxygen uptake, corrected for the influence of body size. These findings are substantial in agreement with the observations of several previous investigators [5, 18, 43—46, 48].

Astrand [5] for instance, found no certain relationship between a hemoglobin concentration within the normal range and maximal oxygen uptake (ml/kg × min) in male subjects (schoolboys and male students) as well as in female subjects (school girls and female students). Saltin et al. [48] observed almost no change in hemoglobin concentration after bed rest or training, while maximal oxygen uptake varied considerably.

Furthermore, experimentally induced changes in the hemoglobin concentration have been found to influence the maximal oxygen uptake only to a minor degree [44—46]. Rowell et al. [45] for instance, showed that a reduction in hemoglobin concentration of 14 per cent due to repeated phlebotomies over an eight days period, gave almost no reduction in maximal oxygen uptake. Saltin [46] found no change in maximal oxygen uptake after thermal dehydration with an increase in hemoglobin concentration of approximately 8 per cent and a reduction in plasma volume of 14 per cent. Similar results have been found after transfusion of one liter of blood [44].

Even in rather pronounced degrees of iron-deficiency anemia, no noticeable reduction in physical performance has been demonstrated [18, 43]. Robbe [43] for instance, found no influence of anemia (individual values as low as 8.2 g/100 ml) on the physical work capacity of pregnant women. Cotes et al. [18] made the same observation in non pregnant anemic housewives (mean Hb 8.6 g/100 ml). Iron medication restored the hemoglobin concentration to a normal value (mean Hb 13.0 g/100 ml) but had no effect on physical performance. The only suggestion of reduced physical work capacity in anemia, has been presented by Sproule et al. [52] who observed lower values for maximal oxygen uptake in anemic men as compared to normal subjects. Whether such a comparison, however was relevant or not, might be questionable. The maximal oxygen uptake was neither corrected for body size, nor measured after the restoration of the hemoglobin concentration to normal. Furthermore, these patients were severely anemic (indivdual Hb values as low as 5.2 g/100 ml) and only a few had iron-deficiency anemia. Pernicious anemia or sickle cell anemia was diagnosed in the remainder of the subjects, and thus, neurological manifestations also might have influenced the physical work capacity.

So far the discussion has been focused on the influence of the hemoglobin concentration on physical work capacity. A similar lack of relationship was revealed when the *hematocrit* was considered. A

highly significant positive correlation with maximal oxygen uptake (liter/min) was found in the total primary material. However when the maximal oxygen uptake was expressed as ml/kg × min, no reproducible significant correlation was demonstrated. The results of the longitudinal analysis as well as the experiments with iron and placebo confirmed this observation.

With regard to the other hematological indices examined, there was no reproducible significant relationship between *MCHC serum iron TIBC and transferrin sat%* and maximal oxygen uptake expressed as liter/min as well as ml/kg × min. This lack of relationship was also supported by the results of the longitudinal analysis as well as the results of the experiments with iron and placebo. These results are in agreement with the findings of Adolfsson et al. [1] who observed no reduction in physical performance of blood donors after repeated phlebotomies which gave a reduction in depot iron.

In order to make a further assessment of the relationship between circulating hemoglobin and physical work capacity the *total quantity of hemoglobin (THb)* was measured in a sample of male and female students (primary material) In a similar way as for hemoglobin concentration, a highly significant positive correlation was found between THb and maximal oxygen uptake (expressed as liter/min) when calculated in the entire sample, both at the first and the sixth examination. However when the correlation analysis was performed in male and female subjects separately this relationship could not be confirmed. The same lack of association was found when maximal oxygen uptake was corrected for the influence of body size (ml/kg × min) both in the entire sample and in each of the subgroups.

Previous workers, however have emphasized the close association between THb and physical work capacity [5 33 50 55] In studies by Kjellberg et al. [33] and Sjöstrand [50] for instance, a highly significant correlation was found between the two parameters. In these studies the physical work capacity was not measured in terms of maximal oxygen uptake but was expressed as the absolute work load at a heart rate of 170 beats per min, according to the method of Wahlund [56] This procedure will only give an approximate estimation of oxygen uptake in liter/min and does not eliminate the influence of body size. Åstrand [5] and von Döbeln [55] also found a close correlation between THb and physical work capacity Although physical work capacity was measured in terms of maximal oxygen uptake in their studies, the correlation analysis was based on data given as liter/min. When correlation for body size was performed by von Döbeln [55] no certain relationship, however could be demonstrated. Thus, most of the discrepancy between the present results and those of previous workers, might be explained by the fact that usually no correction for body size was performed in the earlier studies. When it was performed, no definite relationship could be demonstrated.

In anemic conditions, the ability to deliver oxygen to the tissues is impaired due to inadequate oxygen capacity of the blood [37] The results of the present investigation, however indicate, that the circulatory system is able to compensate for rather pronounced variations in the hemoglobin concentration. Evidence from studies on welltrained athletes indicates that reduced hemoglobin concentration is compensated for by an increase in cardiac output [23] The mechanism by which cardiac output is varied in relation to hemoglobin concentration is unknown, but studies on animals suggest that changes in peripheral resistance may be chiefly responsible [37]

Although the present investigation failed to demonstrate any consistant relationship between hematological indices and physical work capacity it should be noted that severely anemic subjects were not included in our study Further examinations of anemic subjects, however are necessary in order to ascertain the limit below which anemia causes measurable impairment of physical performance.

SUMMARY AND CONCLUSIONS

The present study was undertaken in order to assess the relationship between hemoglobin concentration and other hematological indices, and the physical work capacity

The investigation included repeated determinations of hemoglobin concentration, hematocrit, MCHC, serum iron, TIBC, transferrin sat% total quantity of hemoglobin as well as maximal oxygen uptake (indirect and direct methods) The material consisted of a primary material of 183 schoolchildren and 97 physical education students, a supplementary material of eight female students with low normal/subnormal Hb values and four selected cases of iron-deficiency anemia. All parameters, however were neither determined in all subjects, nor included in all examinations of the same subject.

In the primary material of students (47 males and 50 females) six examinations were performed with approximately eight weeks' intervals during a period of nine months. Two examinations were undertaken in 72 schoolchildren during a period of six months, and only one examination was performed in an additional material of 111 schoolchildren. The female students with low normal/sub-normal Hb values (the supplementary material) were examined with weekly intervals for seven weeks. The selected cases of iron-deficiency anemia were followed for periods of 7 to 26 weeks.

The maximal oxygen uptake increased considerably in both sexes during the nine months examination period, but the hemoglobin concentration increased only noticeably in the female students. With regard to the experiments with iron and placebo it is emphasized that the increase during the nine months period in maximal oxygen uptake was of the same order of magnitude in all subgroups of the primary student material, regardless of the type of medication and also uninfluenced by the initial hemoglobin level. In addition, fluctuations in maximal oxygen uptake and hemoglobin concentration during the same experimental interval were often reciprocal. The same lack of association between changes in hemoglobin concentration and maximal oxygen uptake was also observed in the supplementary material of female students with low normal/sub-normal hemoglobin concentration, as well as in the few selected cases of moderate or mild iron deficiency anemia.

Furthermore, there was no reproducible significant relationship between *MCHC serum iron TIBC and transferrin sat%* and maximal oxygen uptake expressed as liter/min as well as ml/kg × min. This lack of relationship was also supported by the results of the longitudinal analysis as well as the results of the experiments with iron and placebo.

The schoolchildren received no medication as part of the study Experiments with iron or placebo medication were performed in all other subjects. Iron tablets (60 mg iron per day as ferrofumarate) were given to 5 male and 11 female students (primary material) with sub-normal Hb values, and to 21 male and 19 female students with normal Hb values. The remainder of the primary student material (21 males and 20 females) received placebo tablets. Iron therapy either from the start of the experiment or replacing placebo treatment, was also given to the supplementary material of female students, and to the selected cases of iron-deficiency anemia. The daily dose, however was higher (120—180 mg) than used in the primary student material.

Cross-sectional correlation analysis revealed a highly significant positive association between *hemoglobin concentration*

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TWIN REGISTRIES IN THE STUDY OF CHRONIC DISEASE

With Particular Reference to the Relation of Smoking to Cardiovascular and Pulmonary Diseases

Report of an International Symposium in San Juan, Puerto Rico
1—4 December 1969

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STOCKHOLM 1971

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Contents

1 Introduction

For some years a program of research into the effects of tobacco on health has been carried out on Swedish and U S. twin registries This research has been under the auspices of the Karolinska Institute of Stockholm and the National Academy of Sciences, National Research Council of the U.S.A. Particularly the Swedish research has been focused on cardiovascular and pulmonary effects of smoking. The methods used have included questionnaires, clinical evaluations and mortality studies Although n many respects these studies in twins were in accord w th previous epidemiologic evidence, in other regards they did not confirm earlier results Interpretation of the discrepancies is difficult sinc the number of twin pairs studied was small.

Appropriate extension of this research using the Swedish and U S twin registries is being considered It therefor seemed important to evaluate how such twin studies would compare with and supplement data from more commonly used epi demiologic investigations. With this background conference to provide additional advice on the most effective use of the available resources was convened Organization of this meeting was the responsibility of the Department of Environmental Hygiene of the Karolinska Institute, Stockholm. The meeting was financially supported as a special project of the Council for Tobacco Research, U.S.A. It was held in San Juan, Puerto Rico, December 1 through 4, 1969 The meeting was opened by brief statements from L T Friberg of the Karolinska Institute and by Z. Fejfar of the World Health Organization (WHO) At the meeting E. P Radford was elected Chairman, and F H. Epstein was elected Vice Chairman, and Z. Hrubec and R. Cederlof were elected rapporteurs

The participants were given the assignment to review previous work, to consider designs for new studies in twins and to suggest methods for the effective conduct of these investigations Working papers submitted prior to the meeting by the par ticipants formed the basis for the discussion and the final report.

The report as prepared at the symposium and after preliminary editing was reviewed by all the participants The final editing was done by committee consisting of R Cederlof, F H Epstein, L T Friberg, Z Hrubec, and E P Radford

2 Smoking studies – specific considerations

A variety of retrospective and prospective epidemiologic studies have documented an excess morbidity from a number of diseases among cigarette smokers[1,2,3,4]. For some diseases, e.g. lung cancer and chronic bronchitis, the excess among smokers is so marked and so consistently found that cigarette smoking is considered of causal significance. For other diseases, e.g. cardiovascular diseases, the documented excess among smokers is less marked and has not appeared consistently in all populations. A question has been raised about the inconsistent findings which had led to an unwillingness to accept a causal role for cigarette smoking in relation to these latter diseases. The question is whether smokers and nonsmokers are self-selected groups and therefore not comparable with respect to factors that are important in the etiology of these diseases.

Numerous differences have been found between smokers and nonsmokers. Among other things smokers are more often young, mesomorphic, male urban dwellers who consume more alcohol, vary in marital status, and are under more social stress than nonsmokers[5,6,7]. On the one hand it is implied that smokers are constitutionally different from nonsmokers. For any specific genotype, a smoker might be no more likely to develop disease than a nonsmoker but certain genotypes could become smokers more readily and, independently of smoking, develop disease more frequently. On the other hand there is the position that, regardless of genotype or other (environmental) factors smokers will develop more disease than nonsmokers. To date, for most diseases, it has been difficult to accumulate clear-cut evidence to refute or confirm either of these hypotheses. There are few data, moreover to evaluate the intermediate but vastly more complex hypothesis that disease results from interactions between smoking, genotype and other environmental factors.

It is manifestly impossible to design and implement the theoretically desirable study of classifying individuals by genotype and environmental background, randomly assigning subjects within each class to graded levels of smoking exposure, and then observing each of the cohorts uniformly for development of disease. Designs for alternative studies must therefore be sought. The recent work of the joint Swedish—U.S. twin studies has demonstrated the feasibility and unique value of epidemiologic studies on large samples of the only genetically identical individuals available in human populations—monozygotic twin sets.

An additional advantage of studying monozygotic (MZ) twins is that not only are both members of the set genetically identical but, even within smoking discordant sets, they tend to share a much more comparable environment than smokers and nonsmokers in general. Furthermore, data on dizygotic (DZ) twins, although no more genetically similar than ordinary sibs, provide useful comparison groups matched for age, and sex, and with a tendency to have common environments.

3 Twin studies – methodology and applications in smoking research

3.1 WHO REVIEW OF THE USE OF TWINS IN EPIDEMIOLOGIC STUDIES (1965)

At a meeting sponsored by the WHO in 1965[8] the possible contribution of twins in epidemiologic studies of chronic diseases was reviewed. In contrast to investigations of rare conditions with a strong genetic predisposition, a varying number of environmental factors are involved in chronic conditions such as arteriosclerosis, ischemic heart disease or cerebrovascular lesions. The WHO group felt that twin studies offer another useful tool for the epidemiology of the chronic conditions and may assist in estimating the magnitude of the genetic and environmental components respectively and in differentiating the many elements of the latter.

The WHO survey of existing twin studies in the world showed that large unselected twin populations suitable for epidemiological investigations of chronic diseases were available in Scandinavia and in the U.S.A. The report then considered methodologic problems of the twin studies relating to the determination of zygosity, sampling techniques, organization and maintenance of twin registries, and analysis and presentation of results to assist comparison between existing and future twin studies.

The WHO report outlined two main kinds of twin studies: those aiming to compare the occurrence of disease in representative pairs of MZ and DZ twins, and twin controlled studies in which the two members of a genetically identical pair are exposed to differing environmental influences. The latter type was considered as providing the possibility for assessing the causal relationship between environment and disease. The effect of cigarette smoking on mortality and morbidity of MZ twins who differ in smoking habits (smoker and non-smoker) was used as an example.

The group stressed the need for samples of considerable size in order to use the discordant twins for comparative analysis of environmental differences in chronic conditions. Collaborative investigations of course require standardized methods beginning from the planning stage until the publication of result and the WHO was considered as a useful agency for promoting and coordinating such studies at an international level.

3.2 THEORETICAL MODELS IN TWIN STUDIES

3.2.1 Uses of twin samples

The special feature of twin samples in medical research is that twins provide unusual opportunities for the control of genetic and environmental variables. Such control is impossible to achieve with singletons to the degree possible with twins, mainly because members of MZ pairs are identical genetically. In addition, members of twin pairs tend to share many environmental variables. There is no generally accepted methodology in the design of twin studies and there are many complex possibilities. In epidemiologic investigations it is generally advisable to verify first that the data being analysed do indeed reflect the relationship of the suspected agent with the disease of interest as would an uncontrolled study of singletons. This can be evaluated, for example, by considering the twins as individual smokers or nonsmokers and comparing the disease experience of these groups. Data analysed in this fashion are discussed in 3.5.

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Evidence sought	Conclusions, if any	Restrictions on the conclusion
One or more pairs discordant for D_1	"D_1 not entirely determined by heredity"	Valid only for the type of disease represented in the discordant pairs
One or more pairs discordant for S_1	"S_1 not entirely determined by heredity in people with D_1"	Affirmative conclusions not justified
One or more pairs discordant for D_1	"Susceptibility to D_1 after exposure to E_1 not entirely hereditary"	
History in affected partners only of exposure to factors E_1 ... E_n	E ... E_n are possible etiological agents in D	Associations likely to be by chance, or to be indirect rather than causal
Reliable association, within pairs, of D_1 and E_1	E_1 sometimes causes D_1	Associations may be indirect and not causal
Reliable associations, within pairs, of E with D_1 ... D	E_1 sometimes causes D_1 ... D	
Reliable association, within pairs, between course of the disease and E_1	E_1 sometimes influences the course of D_1	
Concordance rates reliably increased over expectation: MZ> DZ> chance	Heredity is significant factor in D_1	See Text Valid only for the population under study
High concordance rate for D' still higher rate of D in partners of D' cases	Factors responsible for D also accentuate susceptibility to D"	D' or S_1 may not be genetically determined
High concordance rate for S_1 high frequency of S_1 in healthy partners of cases with D_1	S_1 is mark of susceptibility to D_1	Conclusions depend on assumed model of pathogenesis and, for quantitative variables on scale of measurement

In the next section each of the above models is discussed specifically

3.2.2 Exclusion of complete genetic determination

The interpretation of twin data in terms of complete genetic determination of disease can be formulated in a single description which will be called Model I (Table 1) When a hypothesis of complete genetic determination is in question, family data may not be decisive and a test is then appropriate in MZ twins In principle, a single discordant MZ pair and in practice some small number of discordant pairs, should suffice to exclude the hypothesis This kind of analysis depends only on the fact that all differences between MZ twin partners are the result of environment or measurement error Failure to find discordant pairs does not establish the hypothesis because all pairs may be concordant for the critical environment factor. If however an intrauterine cause of disease seems unlikely and many observed twin pairs are all concordant, genetic determination can be regarded as tentatively sustained pending confirmation with biochemical or anatomical evidence

TABLE 2 Measures of Risk and Risk Ratios Used to Evaluating the Role of Smoking in Disease Q

	One individual in each pair		MZ smoking discordant pairs		DZ smoking discordant pairs	
	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker
Rate (risk) of disease Q	P_{st}	P_{ns}	P_{ms}	P_{mn}	P_{ds}	P_{dn}
Risk ratio	$R_s = \frac{P_{st}}{P_{ns}}$		$R_m = \frac{P_{ms}}{P_{mn}}$		$R_d = \frac{P_{ds}}{P_{dn}}$	
Comparison	Between pairs ⟷		Within MZ pairs ⟷		Within DZ pairs	
	Between pairs				Within DZ pairs	
Type of analysis	A		B		C	

Regardless of zygosity

an inconclusive result is about 0.25 and after 10 years of follow-up it will be about 0.17. These evaluations are specific for a restricted age range and do combine the data for males and females. They assume that the comparison is made between MZ and DZ groups of equal size and that smoking discordance is defined in an ever smoked, never smoked dichotomy. Combining data from different registries would reduce the risk of an inconclusive result but is subject to the special methodologic problems described in 3.3.5.

3.3.2 Pairing

The general form taken by a comparison of disease experience for smoking discordant pairs can be expressed in the following 2×2 Table 3 which is an application of Model II in 3.2.3 above.

TABLE 3 Disease by Smoking Status in Smoking-Discordant Pairs

		Smoking member with disease	Smoking member without disease	
Non-smoking member	With disease		b	a+b
	Without disease		d	+d
	Total	+	b+d	N

There are a+b+c+d twins of which a are concordant disease positive and d are concordant disease negative. For b of the pairs the nonsmoking member is positive, while c of the pairs are disease discordant in the reverse direction. This form of analysis differs from that of Model IV in 3.2.5 in that it does not attempt to estimate the effect of genotype hence it does not require knowledge of the concordance rate for disease in unexposed pairs.

An estimate of the risk for smokers relative to nonsmokers often used is $(a+c)/(a+b)$ with confidence or probability limits obtained by assuming independence, i.e. by disregarding the pairing. If a is greater than random expectancy the independence assumption is not true. The general question of pairing in 2×2 tables is a familiar one in statistics[12,13] although the subject has generally not been dealt with in terms of relative risk computations.

3.3.3 Quantitating dose and response

Between-twin pair comparisons utilize multiple smoking classes i.e., nonsmokers, light, moderate, and heavy cigarette smokers, etc. A continuing increase or decrease in risk with increased or decreased amount of smoking has had probative value. To date the within-pair comparisons used

primarily dichotomous groupings, i.e., between smokers and nonsmokers or between low and high intensity smokers. Except for concordant nonsmokers, all other twin pairs are discordant to some degree. Extensions to multichotomous comparisons would appear possible.

3.3.4 Other variables in within-pair comparisons

In comparing the relative risk of smoking in the different twin types, i.e., MZ versus DZ, or for within-pair and between-pair comparisons, it is important to examine the comparability of these groups with respect to other variables, particularly age. A disparity between age groups in the increase in risk of CHD with smoking, unless adjusted for, could lead to a possibly incorrect conclusion, namely that when genetic variation is reduced, the apparent relative risk of smoking is decreased or even eliminated.

When such lack of comparability is found, several statistical procedures are available for minimizing its effects. One can evaluate only age-matched pairs although this does not use all the twin material. Age standardization avoids discarding pairs, but results may be dependent on the standardization method. Least squares procedures avoid these difficulties, but are time consuming, unless computers are available. All three procedures assume that the effect of age on relative risk is the same for both twin types. Newer statistical procedures of the type developed for the Halothan Study[14] relax even this assumption and may be more appropriate.

At least two types of comparison seem necessary for other variables. For only a simple dichotomy on a second variable, say "high" and "low" alcohol consumption, one possible comparison is that of relative risk for smoking-discordant pairs who are concordant-high with those who are concordant-low. The second is that of relative risk in variable-discordant pairs in which the smoker is high with those in which the smoker is low. The ability to conduct such studies is limited by the number of available cases. The calculation of confidence or probability limits will indicate when the data are too variable to support strong conclusions.

3.3.5 Comparability of methods and pooling

New twin registries can serve at least two important functions. To the extent that the results of replicated studies are in agreement, the precision of estimates can be increased by pooling the findings. If increased precision is the objective, it is necessary that all registries employ common procedures, so that results may be compared and combined. Common protocols and standardization of procedures may be required, although each group may still be free to develop ancillary studies that do not interfere with the common core.

Increased precision of results through standardization and through pooling of data does not allow independent verification through separate studies, since uniform procedures may be adopted which later experience might prove to be inappropriate. In practice it may be necessary to strike a balance between the two objectives, with all registries consulting each other and thus assuring a minimum degree of comparability, but with each proceeding independently.

If pooling is indicated, appropriated methods of pooling should be considered carefully. Simple addition of the results of several studies can lead to erroneous conclusion. The experience of the American pooling project for longitudinal studies of CHD is relevant[15] as well as the various theoretical procedures developed for such problems.

3.4 LIMITATIONS AND REQUIREMENTS OF TWIN METHODS

Restrictive assumptions necessary in the analysis of twin data have been mentioned above in connection with the models to which they applied. The following additional problems need to be considered in twin research.

First, difficulties of interpretation include the special nature of a twin population. The distribution of DZ twin births is unequal among socioeconomic classes, among birth ranks, and among maternal ages. Of even greater significance may be the bias introduced by the requirement of survival or health of both partners to the time of registration or collection of data, especially if this

occurs at a mature age. This requirement implies a relatively healthy state not only in the index case, as in other epidemiological studies, but also in his twin partner, and the last requirement adds further assurance of health. However the consequences of this selection may not be too serious in the analysis of *differences* between MZ and DZ pairs, as the possible bias is likely to be similar in both zygosity groups. When one partner is not readily available the limited availability of data will introduce di verse biases that are very difficult to evaluate. Finally samples of MZ pairs discordant with respect to some exposure factor are of particular interest in epidemiologic studies but such samples may be atypical of twins as a whole.

Practical difficulties include the restricted size of the twin population and its wide geographic dispersal, as compared with unrelated individuals who can be studied in a small geographic region. The usual problems of obtaining contact and cooperation might be increased when members of a twin pair must both be found before either can be included in a study In practice, however twins have been found to be very cooperative. A unique problem of twin research is the need for zygosity diagnosis, which should have a level of accuracy at least commensurate with that of the data to be obtained.

3.5 APPLIED TWIN RESEARCH IN SMOKING AND HEALTH IN THE SWEDISH AND THE U.S. REGISTRIES

3.5.1 Materials available for further research

3.5.1.1 The Swedish Registry

The Swedish Registry was compiled during 1959—61 and consists of 10 320 pairs for whom zygosity and smoking history could be established. It covers about 73 percent of all pairs of the same

TABLE 4 Number of Twin Pairs in the Swedish Twin Registry by Sex, Year of Birth Smoking Status and Zygosity

Year of birth	Smoking status[1]: Concordent smokers: Male	Female	Smoking[2] discordent: Mal	Female	Concordant non-smokers: Male	Female	Total: Male	Female
				Monozygotic				
1886—1895	73	5	60	8	50	109	183	202
1896—1905	194	15	64	31	66	351	324	397
1906—1915	318	68	83	103	119	461	520	632
1916—1925	355	165	105	151	141	418	601	732
Total	940	251	312	293	376	1 419	1,628	1,963
				Dizygotic				
1886—1895	77	3	70	29	59	341	206	373
1896—1905	171	26	179	99	106	663	556	788
1906—1915	494	101	306	243	199	867	999	1,211
1916—1925	648	220	338	386	191	813	1,177	1 419
Total	1,490	350	893	757	555	2.684	2,938	3 791
				Unknown Zygosity				
Total	106	17	57	41	40	162	203	220

[1] Smoking status unknown in 204 pairs of whom 65 MZ, 123 DZ and 16 zygosity unknown.

[2] Only pairs in which one twin has never smoked and the other is current or former cigarette, pipe or cigar smoker are included in this category In some studies on the Swedish Registry different definitions of smoking discordance have been used, and thus the numbers shown here may not correspond with other published data.

sex born in the country between 1886 and 1925 and still alive at the time of compilation. Since that time about 1,200 twins have died. A detailed description of the compilation procedure and the *demographic structure of the twin series has been* published[14]. A summary of relevant numbers in different categories is in Table 4.

In the Swedish Registry data were collected using three questionnaires, by two clinical studies on subsamples and by observing mortality. The questionnaires were mailed to the entire sample. Information was sought about zygosity, smoking history, *anginal and respiratory symptoms*, as well as a variety of socioeconomic variables and other environmental and biological factors. Of the clinical studies one used about 200 smoking-discordant pairs and the other about 100 pairs discordant for signs of CHD. Mortality was studied by means of death certificates for the entire deceased group supplemented by hospital and other records for a subgroup of smoking discordant twin pairs.

3.5.1.2 The U.S. Registry

The U.S. Registry is operated by the National Research Council, Washington, D.C., and contains white males born in 39 out of 48 continental states during the years 1917—1927 of whom both members served or serve in the U.S. Armed Forces. The *compilation procedure*, based on matching birth records with the Veterans Administration (VA) index files, has been described in detail by Jablon et al.[17] Over 50 000 pairs of twins were so screened and in about 16 000 pairs both members were identified in the VA index. Questionnaire responses, which allow zygosity determination, classification with respect to smoking and medical symptoms, were obtained from 4,008 pairs. Of *these pairs there were 1 876 MZ, 1,999 DZ*, and 133 of unknown zygosity. Among the MZ pairs 977 were concordant smokers and 472 were smoking discordant, other counts are given in Table 5. The questionnaires used in the U.S. study was a translation of the Swedish questionnaires. Except for minor changes the same information was sought as in the Swedish studies.

3.5.2 Summary of published work

This entire section is a summary of earlier publications prepared by the Swedish and U.S. investigators participating in the conference (ref. 18—29).* In the subsequent discussion the evaluation of findings on individuals is referred to as the *A-analysis* which was defined in 3.2.1. Comparisons of smokers and nonsmokers in smoking discordant twin pairs according to Model II (3.2.3, 3.3.1) will be referred to as the *B-analysis*.

3.5.2.1 Respiratory disorders

Questionnaire responses in the Swedish and the U.S. sample have been used to obtain a classification of cough and bronchitis following methods developed by the British Medical Research Coun

TABLE 5. Number of Twin Pairs in the NRC Twin Registry by Year of Birth and Smoking Status and Zygosity

Year of birth	Smoking status: Concord. smokers	Smoking discord.[1]	Concord. nonsmok.	Total
		Monozygotic		
1917—1920	182	108	116	406
1921—1924	411	197	154	762
1925—1927	384	167	157	708
Total	977	472	427	1,876
		Dizygotic		
1917—1920	223	195	84	502
1921—1924	384	298	127	809
1925—1927	352	225	131	688
Total	959	718	342	1,999
		Unknown Zygosity		
1917—1920	18	8	2	28
1921—1924	28	26	9	63
1925—1927	17	16	9	42
Total	63	50	20	133

[1] Same criteria used as shown for Table 4, footnote 2.

*The mortality data and the study by Liljefors on CHD were only presented in preliminary form at the meeting but have since been published in more complete form. The numbers presented in this section refer to the published data.

cil[30] A marked association between smoking and respiratory symptoms in the A analysis and also in the B-analysis has been noted In the B-analysis the association is evident both for the monozygotic twins, for males and females, and for all age groups For the age and sex groups that were comparable, no difference of importance could be seen between the U.S and the Swedish results. Analysis of the symptom "cough according to Model IVa, indicated that nonsmokers with a twin partner who coughed had a risk of "cough at least as high as that of smokers whose twin partner did not report cough

The association between smoking and symptoms of chronic bronchitis was evident in the clinical study of smoking discordant twins. Further in that study an impairment of the lung function associated with smoking could be demonstrated both in MZ and DZ twins. For example, in MZ pairs the mean value of forced expiratory volume for 1.0 seconds (FEV 1.0) was 0.20 liter lower for the more exposed partners as compared to the less exposed. This difference is highly significant statistically There was also an association between the amount of cigarettes consumed and different tests of airway resistance and uneven ventilation

Lung cancer was also clearly associated with smoking in an A-analysis of the data for males in the Swedish Registry No such association was found for females. The number of smoking discordant pairs is too small to allow conclusions in the B-analysis

3.5.2.2 Coronary heart disease

In the A analysis the Swedish as well as the U.S questionnaire studies showed an association for males between smoking and "angina pectoris defined operationally from questionnaire responses using modification of the method developed by Rose[31] The Swedish male smokers reported symptoms 1.[illegible] times more frequently than nonsmokers. The same association was found in the U.S study When a B-analysis was carried out no association with smoking was found for the MZ twins either in the Swedish or in the U.S. data.

For DZ smoking discordant twin pairs an association with smoking could be seen in the U.S. study but not in the Swedish study

The analysis of the Swedish data evaluated according to Model III (3.2.4) indicated that in the older age groups MZ males had a higher coincidence rate than the DZ males MZ females of all ages had a higher coincidence rate than DZ females The observed coincidence rate for MZ twins was well above the expected rate

The Swedish clinical studies give further support to the trends found in the questionnaire investigations. There was no evidence that smoking was associated with signs of CHD in DZ or in MZ pairs. Further, in the Swedish clinical study on smoking discordant pairs, no within-pair difference was found in regard to serum cholesterol or triglyceride levels. On the other hand a lower systolic and diastolic blood pressure and a tendency to lower body weight and lower skinfold thickness was found among smokers than among nonsmokers In the clinical study of twin pairs discordant for CHD no meaningful differences in mean blood pressure or cholesterol levels were found between the affected and well members of these pairs. There were 14 MZ twin pairs and 10 DZ twin pairs discordant with respect to myocardial infarction. There was a tendency for twins without infarction to be more physically active in their leisure time activities but not at work. They were not as highly motivated to achievement at work, and conflict in work situations was not reported as often as by their partners with infarction.

The clinical twin studies do indicate a presence of genetic factor in coronary heart disease and in variables which have been found related to it. For instance, blood pressure, serum cholesterol, and triglycerides tend to be correlated between the members of pairs and in addition post-exercise ST depressions of the ECG were the same in the twin pairs regardless of smoking. In the second clinical study of pairs with angina there was a higher concordance rate for signs of CHD in the MZ group than in the DZ group The difference was not statistically significant in this limited series, but concordance rates of the same magnitude have

recently been reported by Harvald and Hauge[32]

Further analysis of the Swedish and U.S. questionnaire data tried to identify factors other than smoking associated with "angina pectoris" Variables dealing with alcohol drinking, diet, physical exercise, social characteristics, and occupational adjustment were evaluated by determining the relationship of each variable with angina pectoris in a B-type analysis when smoking exposure was controlled.

Among the MZ twins in this analysis significant differences in angina pectoris rates were found only for drinking of alcohol. Among the DZ twins the positive members of pairs discordant for alcohol drinking, change of place of employment, and occupational adjustment had significantly higher rates of angina pectoris

3 5 2 3 *Mortality*

The mortality data from the Swedish twin registry up to fall 1968 have been analyzed in smoking discordant monozygotic and dizygotic twin-pairs (B-analysis) A statistically significant higher mortality among smokers was shown in dizygotic male pairs, born 1901—1925 but not in monozygotic male pairs, or in females of either zygosity No increased mortality was apparent among smokers in the older age-groups (1889—1900)

The excess mortality of smokers in the male dizygotic group is due to several causes and it is not possible to associate it particularly with any specific disease Of the total number of 21 excess deaths among the smokers three were caused by CHD Three cases were due to cerebrovascular disease, 4 due to lung cancer 5 due to other forms of cancer 5 due to suicides or accidents and one due to other causes

3 6 EVALUATION OF TWIN STUDIES

There is no doubt that the development of twin methodology as seen in the Swedish and the U.S. registries has added an important tool for studying the effect of smoking on health. The twin data continue to support a close relation of smoking status to respiratory symptoms in smoking discordant twins The finding confirms the results from other epidemiological studies and strengthens further the evidence of a causal relationship between cigarette smoking and such symptoms One analysis has illustrated also the potentiality of twin studies for investigating interactions between the genotype and the environment. This application should be further explored in future studies with elucidation of the relevant theoretical models and testing of promising hypotheses The numbers available appear to be a limiting factor in studies that require particular grouping for age and sex and other relevant characteristics.

As far as CHD is concerned a positive correlation between chest angina and amount of cigarettes smoked has been found both in the Swedish and the U.S A-analysis in which one individual member was selected from each twin pair However, no such relation within MZ pairs has been found in the B-analysis of smoking discordant pairs This finding in the two questionnaire studies was consistent with the results of the clinical study by Lundman[24] The present material does not allow a clear decision as to whether the explanation of the apparent disparity between the results of A and B-analysis lies in an insufficient number of study cases, or whether the relationship really disappears upon control of genetic factors There are of course obvious difficulties in diagnosing CHD from the chest pain questionnaire There are similar problems when other diagnostic methods and procedures are used in epidemiological as well as in clinical studies—as discussed in another part of the report.

On the other hand, the data although preliminary suggest that when smoking is controlled several factors such as alcohol drinking, change of place of employment and occupational adjustment are associated with angina pectoris. Such factors have not been extensively evaluated together with smoking in relation to CHD In view of the preliminary nature of the findings on the association of these factors with angina pectoris alternate analyses of these data will be undertaken.

The prospective seven year mortality data show a relationship between smoking and overall mor

tality in the younger age groups in the smoking discordant DZ twin pairs, but not in the MZ pairs. The numbers although small are suggestive, and it is obvious that follow-up of the groups in twin registries should provide further information on this important matter

Several other interesting findings were noted which could not be statistically evaluated because of the insufficient numbers studied. They nevertheless suggest the potential of twin registries for investigating the relation of smoking and other variables to health status and particularly to CHD and chronic respiratory disease as well as to the measurable abnormalities of the host known to be predisposing to this condition. Particular emphasis should be placed on the relevance of studying the living habits of smoking concordant and discordant twins.

It is clear that the twin studies to date, taken as a whole, have produced important findings. Clarification of the important issues raised in these studies fully justifies a continuing effort in the use of existing registries, and extension of this work to new twin groups in the future

4 Epidemiological considerations

4.1 GENERAL ASPECTS

4.1.1 Methods of data collection

In this section the demands for adequate numbers of subjects and the need for information of high quality are discussed. To accommodate the range of demands which are to some extent mutually limiting, field studies may be subdivided into those in which large numbers of persons are examined by methods that are relatively simple and quick to administer and those, in which smaller subsamples of the total population are studied by clinical methods adapted for epidemiological purposes to be used in the field. The latter kind of study is half way between a total population study and a detailed clinical investigation carried out in a hospital setting or physicians' offices.

In large-scale field studies information on symptoms and attitudes, may be elicited economically on a very large number of persons by mail questionnaires. The disadvantage lies in the lack of control over the comprehension of the forms and accuracy in their completion. The methodology of questionnaire construction has been discussed extensively in the literature, especially with respect to sociological and marketing applications[33, 43]. It is generally accepted that careful pre-testing is required. Questionnaires administered by trained interviewers are more expensive but the data obtained are more reliable, and more complex questions may be asked. At the other end of the spectrum are medical examinations which include detailed clinical evaluation of the subject by physicians. Intermediate methods consist of tests carried out by trained technical personnel.

A useful tool for morbidity detection is the examination of work or school records for absences due to cardiac, respiratory or other illness, or hospital admissions for similar reasons. The value of such record searches, of course depends on the completeness of recovery of the pertinent information. This technique has been used effectively in Japan and England and should in theory, at least, be valuable in Sweden, where personal health records are well maintained. At the present time such information is not likely to be complete in the U.S.A., except from hospital records. In a study of hospital records of myocardial infarction cases suspected from field examinations, Keyes[35] found 93 percent of the needed documentary evidence.

There is a great need in epidemiological research to extend to field use the more complex clinical and laboratory methods hitherto feasible only in institutional settings. Inevitably, such methods will not be readily applicable to large numbers of subjects. Two solutions present themselves. Suitably stratified subsamples of the total target group may be examined in specially equipped field laboratories, or the subjects may be brought to the hospital or physicians' offices for examination. Under any circumstances, consideration must be given to the need to collect data so that comparability is made possible through good standardization of procedures between observers and centers. In this connection, it should be stressed that quite complex, mobile equipment can often be used even under quite primitive working conditions and in remote areas.

4.1.2 Incidence versus prevalence studies

Scientific considerations — There is no question that incidence studies are preferable to prevalence studies if the aim is to prove that a factor or a constellation of factors precede and, in fact, predict the onset of manifest disease. It may be stated in general that the presence of an association between a factor and a disease, detected in a preva-

tality in the younger age groups in the smoking-discordant DZ twin pairs, but not in the MZ pairs. The numbers although small are suggestive, and it is obvious that follow-up of the groups in twin registries should provide further information on this important matter

Several other interesting findings were noted which could not be statistically evaluated because of the insufficient numbers studied. They nevertheless suggest the potential of twin registries for investigating the relation of smoking and other variables to health status and particularly to CHD and chronic respiratory disease, as well as to the measurable abnormalities of the host known to be predisposing to this condition. Particular emphasis should be placed on the relevance of studying the living habits of smoking concordant and discordant twins.

It is clear that the twin studies to date, taken as a whole, have produced important findings. Clarification of the important issues raised in these studies fully justifies a continuing effort in the use of existing registries, and extension of this work to new twin groups in the future.

ing effects should include all smoking discordant MZ pairs and a suitable number of DZ pairs

The data on smoking exposure in the Swedish and U.S. studies are based on questionnaires related to smoking habits at the time of study More extensive information representative of life time habits (amount, duration, and mode of smok ing) might allow a reclassification of some pairs, who now are considered smoking-concordant and who might turn out to be more or less discordant, a finding which could strengthen the power of the analysis. The inclusion of concordant smokers as well as concordant nonsmokers will also make it possible to assess smoking effects on individuals constitutionally unrelated to each other by making between pair comparisons Further a study of *smoking concordant pairs permits investigation* of other risk factors *within* pairs where the effects of smoking have been controlled.

If a study of the total number of twins is not possible for economic or other reasons subgroups discordant for specific exposures can be selected. Pairs discordant as well as concordant for certain diseases may provide valuable samples for specific studies.

There are already indications of n association between smoking and disease in women[39] Women began smoking later but their smoking be havior now approaches that of men For these reasons studies in women should be specifically in-cluded Since chronic diseases tend to have their origins early in life, it is most des rable to start ob-servations at that time in order to evaluate the evolution of genetic en ironmental interactions among predisposing factors.

A study of mortality is of great importance The cause of death on the death certificate should be supported by additional reliable information A valuable extension of the mortality studies may be clinical examinations of partners of twins who have died, in order to detect any differences in disease prevalence between surviving MZ and DZ co-twins Such examinations should preferably be performed within a year from the time of death. In some circumstances, retrospective data on de eased twins may be worth examination.

Finally comparison of twins reared or living together and apart is valuable in the study of en-vironmental influences The various and possibly confounding environmental factors should be evaluated together with an assessment of smoking effects. In practice twins separated in early child hood may not be easy to find

4.2.3 The need for studies in different populations and subgroups

Although the evidence relating cigarette smoking to pulmonary disease has been established in a wide variety of settings, the observation that there is a statistically significant association of cigarette smoking and increased mortality and morbidity f om coronary heart disease n man has been drawn principally but not exclusively from investigations n the Un ted States and the United Kingdom. It should not be assumed that such associations are to be found under all environmental and genetic con-ditions A preliminary report from an international collaborative study suggests that the relationship between smoking and the incidence of coronary heart disease is much less apparent n some countries than in others[33] There is a need for smoking and heart disease studies in widely dif ferent populations, in racial and ethnic groupings, and in contrasting socio-economic, occupational, and environmental settings This applies to twin studies s much as to epidemiological studies in general

Any advance in the identification of susceptible individuals would mark a most important advance n the study of environmental agents. This would provide a marker which would allow modification of the natural history of the disease. Accordingly there is also the need for identification of smoking subgroups within populations which may be par ticularly sensitive or resistant to coronary heart disease using factors such as parental disease history serum lipid levels levels of physical ac tivity obesity body build (somatotype) or occu pation. Any findings of synergism from combi-nations of these markers would further enhance the identification of the most susceptible and the most resistant persons.

5 Methods and Criteria for Measuring Environmental and Other Influences in Smoking Studies

5.1 METHODS FOR EVALUATING RISK FACTORS COMMON TO ALL STUDIES OF CHRONIC DISEASE

5.1.1 Classification of risk factors

A number of risk factors are pertinent to studies of medical effects of smoking regardless of the specific diseases under investigation. On the one hand, there are influences which may be considered to be primarily environmental, or "extrinsic" such as smoking itself. On the other hand, there are influences which may be referred to as constitutional or intrinsic. The use of the terms extrinsic or intrinsic is perhaps preferable because they do not imply specific mechanisms. In the discussion of risk factors that follows, it is clear that their division into these two categories is arbitrary at best.

Difficult though it is to obtain valid life-time histories of smoking, the situation is more favorable than for say nutrition, which is notoriously difficult to assess. For the purpose of weighing the relative importance of constitutional and environmental factors, the validity of the measurement may matter less than its reproducibility. In the case of diet, answers to a questionnaire may not adequately reflect what a person actually eats, but if the errors are systematic the method may be adequate for comparing groups of subjects.

Data on risk factors should be collected in such a manner that they can be quantitatively classified in several ways. Thus decisions regarding "high risk" or "low risk" groups may be left until the data are in hand rather than defining them fully at the outset. If numbers permit, it may be possible to separate the risk groups into several grade levels, from low to high, and include comparison of the extremes which are clearly different from each other.

5.2.1 Extrinsic risk factors

5.1.2.1 Smoking Types and Sources of Materials Smoked

Cigarette smoking is the principal risk factor under investigation since it is now clearly indicated from many studies that pipe or cigar smoking constitutes a different type of risk. The kinds of cigarettes used may be different depending on the presence of filters, length of the cigarette and source of tobacco. The brand of cigarette should be specified if possible. The use of questionnaire methods in evaluating these factors is reasonably satisfactory and no suitable alternative is evident.

Inhalation and other factors affect absorption of smoke constituents. Apart from oropharyngeal changes, the effects of smoking on the cardiorespiratory system can be assumed to be related to the amount of smoke which comes in contact first with oropharyngeal, bronchial or alveolar surfaces of the airways and lungs, and through subsequent oral, alveolar or intestinal absorption with the cardio-vascular system. There have been few efforts to measure the amount of materials absorbed into the body from smoking, but a dose-response relationship is likely and ought to be looked for. An additional factor of exposure is the length of cigarette smoked, since the temperature, CO and "tar" content of smoke depend on the length of butt remaining[40]

Inhalation is thought to be a risk factor associated with smoking. At the present time the chief method of measuring inhalation is by question-

nare, but this technique is not believed to be very accurate.

A method that may prove useful in defining current smoking exposure to the lungs is the measurement of blood carboxyhemoglobin, or alternatively expired air carbon monoxide under controlled conditions. Carbon monoxide measurements reflect only the amount smoked within the past few hours but because daytime smoking rate is often constant, absorption of smoke can be quantitated in relation to smoking habits such as degree of inhalation.

Methods available for assessing the age of beginning smoking and subsequent duration are by questionnaires with the possibility of collaboration from siblings or other relatives. An important measurement is the time of cessation of smoking for individuals not currently smoking.

5 1.2.2 Occupation

It is worthwhile to obtain data on both occupation and possible toxic exposures by questionnaire, so that occupational risk factors can be identified. Methods have been applied by the British MRC Questionnaire in several general population studies[41] with useful results. As many as 10 % of working-age males may have occupational exposure histories with potentially important pulmonary disease risks[42]. These methods are relatively insensitive, that is suspicious occupational exposures may easily be missed.

5 1.2 3 Socio-economic status housing and other extrinsic social factors

Such characteristics have not been considered in many studies as yet and their value in twin populations may be important although their effects may be slight in within-pair comparisons. Inclusion of certain social or economic indicators in questionnaires is valuable as is inclusion of questions related to living space occupancy.

5.1.3 Intrinsic risk factors

5 1 3 1 Age

Age is a highly significant factor in development of cardiovascular and chronic pulmonary disease. In cardiovascular disease, risk factors tend to decrease in importance after about age 60. Thus every effort should be made to study younger populations and this fact may be an important consideration in the use of existing twin registries in which the age distribution is progressively changing.

Age matching of groups of mono- to dizygotic twins may be of great importance as a secondary procedure. Specific attention perhaps should be paid to twin pairs in which morbidity or mortality from coronary disease or chronic obstructive respiratory disease occurs at an early age, since these pairs may be affected by constitutional factors which can be specified, as for example xanthomatosis or α_1 anti-trypsin deficiency.

5 1 3.2 Psychological behavioral aspects

Methods of psychological or behavioral evaluation have been devised[43-49] and results to date indicate the usefulness in epidemiologic studies of ischemic heart disease. Despite the problems of standardization and quantitative measurement of these traits efforts should be made to include them.

5 1 3 3 Ethnic characteristics

Although these factors may be self-evident from the nature of the groups under study there may be the necessity to provide a better genetic marker for racial origin. There are methods available, but their use is probably of limited importance. Some aspects of ethnic characteristics are likely to be extrinsic and relate to nutritional patterns, housing, or reliability of medical information.

5.2 MEASUREMENT OF RISK FACTORS SPECIFIC FOR ISCHEMIC HEART DISEASE (IHD)

A number of environmental factors in persons with varying constitutional traits may be involved in coronary atherosclerosis and myocardial ischemia. In the discussion which follows emphasis is given to the more commonly recognized and more

thoroughly investigated IHD risk factors They include, apart from smoking, nutrition and physical activity serum cholesterol and blood pressure level, glucose tolerance, obesity and body build. These factors, in addition to personality and psycho-social relationships, should be given high priority for inclusion in studies of IHD in twins.

5.2.1 Extrinsic risk factors

5.2.1.1 Dietary habits

An assessment of dietary habits of groups as opposed to individuals, may be worthwhile. Dietary questionnaires or interview techniques are useful, but too crude to differentiate detailed individual differences. Dietary histories are not routinely recommended except for specific purposes in which case a competent nutritionist should devise the interview techniques Diet differences of groups are best studied by diet collections and chemical analyses. The procedure is expensive and time-consuming.

5.2.1.2 Physical activity

Several fairly satisfactory methods of measurement are available to assess the usual physical activity of subjects during work and leisure time Questionnaires aimed at determining usual daily activity have been developed and used with some success [50, 51] Estimates from such methods, despite some of their limitations, will be worthwhile.

5.2.2 Intrinsic risk factors

5.2.2.1 Blood pressure

Casual blood pressure is the usual measurement and it appears to be adequate. Measurements should be obtained by trained observers and under standard conditions[51]

5.2.2.2 Serum cholesterol

The determination of cholesterol can be made on blood specimens without regard to time of the day Regardless of the method chosen it is necessary that each laboratory carries out tests of reproducibility and its performance with a reference laboratory Such standardization can be carried out at WHO International Reference Center for Lipid determination such as the Lipid Standardization Laboratory National Communicable Disease Center Atlanta, USA. Director Dr Gerald A. Cooper Serum can be collected, stored and shipped or a quantity dried on filter paper and thus analysis can be done away from the site of collection. Collaborative studies should maintain a central laboratory where measurements can be checked

5.2.2.3 Blood sugar

Standardized procedures for determining glucose tolerance are available. The determination of a fasting blood sugar and determinations at 1 2 and 3 hours following the dose is most desirable. Simply testing of the blood sugar one or two hours following a meal or a test load is almost as discriminating as a complete glucose tolerance test[51] Serum insulin determinations are also being increasingly included in the evaluation of carbohydrate metabolism.

5.2.2.4 Obesity and body build

It is important to obtain a measurement of obesity but there is no agreement on the best method. The usual method of classifying subjects according to obesity compares their weight to a standard for height, age and sex. Relative weight is far from perfect, but for those who use this technique a recognized table of weight should be used[52] Since these values are readily available, relative weight thus becomes the ratio of the subjects weight to the standard for the appropriate category Some investigators claim an advantage for skin-fold determination to measure obesity[53] Neither skin fold measurements nor relative weight appears to be completely satisfactory Weight gain since age 35 determined from an interview may be a useful gauge of adiposity[54] Constitutionally determined body build has an independent association with IHD In twin studies especially a measure of body build should be obtained. Several methods are available[55]

5.2.2.3 Other factors of optional alu

Serum T glycerides Measurement of triglycerides may add some independent information to serum cholesterol as a predictor and aids in the classification of lipid abnormal ties. The desirability to obtain specimens in the fasting state markedly lowers the usefulness of triglyceride determination in field studies The analysis is more complex and variable than serum cholesterol. Triglyceride determination may be added if facilities and funds are available and if a goal of the study is chiefly to characterize serum lipids.

Electrophor tic Patterns Determination of the electrophoretic patterns of lipids may be a useful procedure in studies of lipid metabolism in twins. Their additional value as a test of risk factors is not yet clear Several methods using impregnated paper and coated glass plates are available[56]

Uric Acid Uric acid levels have shown an association with IHD[57, 58] Its measurement in twin studies may be useful, especially when the levels approach those which are diagnostic of gout.

Xanthomat sis Although routine observation of xanthomata and xanthelasmata should be made, subjects with this disorder are rare Almost all will have markedly elevated serum cholesterol levels and will be detected by this measurement.

Hypothy oid m Latent hypothyroidism is not common in the general population. Inquiry concerning medication with thyroid hormone or other thyroid active drugs should be made. A protein bound iodine determination could be done in suspected cases.

5.3 RISK FACTORS SPECIFICALLY ASSOCIATED WITH CHRONIC PULMONARY DISEASE

Of the risk factors potentially important in development of chronic lung disease those related to place of residence are particularly significant in studies of tw ns especially if international comparisons are contemplated It may be that an important purpose of studies of pulmonary disease in twins w ll be to d fferentiate smoking from other risk factors such as air pollution, climate or genetically significant elements such as allergic pred sposition Because smoking has been found to be such an overwhelming risk factor, the problem of detecting other influences may depend on measurements extending beyond the usual tech n ques including environmental air pollution measurements or a provocative test of pulmonary allergy

5.3.1 Extrinsic risk factors

5.3.1.1 C mmunity air pollution

In existing registries only a small proportion of pairs are likely to have one sibling significantly more exposed to community air pollution than the other Urban rural comparisons used earlier in the Swedish study probably should be extended in further work. The methods are restricted at this time to questionnaire data and information on history of residence Such approaches are neither sensitive nor completely reliable, but they are relatively simple and if they are carefully interpreted, the results may be useful.

5.3.1.2 Alcohol nsumption

A relationship between smoking and alcohol consumption is well-known. In addition, however alcohol consumption may act as a specific risk factor in chronic pulmonary disease because of the effect of alcohol on mechanisms of clearance of viable organisms and inert particles from the lungs[59]

5.3.1.3 Climate zon s

Schoettlin found some evidence of the relevance of this factor in respiratory disease[41] It can be nferred from a location code and may be valuable in relation to the effort required.

5.3.1.4 Exposu e t nfectious agents

This is a risk factor in acute lung disease, and the severity of pertussis in childhood may also be a significant risk factor Study of infectious agents in twins does not seem to be indicated except poss-

6.2.2 Pulmonary symptoms

The British Medical Research Council questionnaire[30] has become the standard, and the methods that have been applied and developed in its preparation are highly regarded by all who have used them in a number of countries. The basic aim of the questionnaire is to determine the prevalence of cough, sputum and shortness of breath using standarized questions While cough during the winter is particularly significant in the United Kingdom, the seasonal distinction may be of little value in other countries.

There is good evidence that in different countries the readiness with which respiratory symptoms, when present, are admitted by those questioned may differ For example, in Japan, the frequency of admitting the production of sputum is substantially lower than one would expect in the presence of other symptoms and pulmonary function impairment, presumably for cultural reasons Burrows and Fletcher[62] have demonstrated in England that persons with equivalent kinds and degrees of respiratory impairment may reply differently to symptom questions than do comparable U.S groups.

6.2.3 Symptoms arising in other systems

The primary emphasis in this report is on cardiorespiratory disease, but in some cases questionnaire evaluation of diseases and symptoms applicable to other systems may be of interest. Detailed discussion of these methods is beyond the scope of this report but qualifying conditions described for all questionnaire methods certainly apply

6.3 PHYSICIANS HISTORY AND EXAMINATION

6.3 1 Ischemic heart disease

Systematic information on validity of the clinical assessment of angina is not available, though correlations are generally good between typical symptoms and coronary angiographic findings. The variability in clinical diagnosis of angina pectoris is wellknown but has rarely been studied systematically

Where one cardiologist, kept unaware (or "blinded) in regard to risk factors, is involved for all comparisons, and where he follows a standard procedure and unambiguous criteria, there may be an advantage derived from security of the diagnosis of classical angina pectoris or myocardial infarction. As an alternative an expert panel of physicians may be used to make a judgment on cardiac symptoms based on systematically collected symptom data and guideline criteria, but they should also be unaware of associated risk factors Physicians judgment of definite myocardial infarction based on the anamnesis, has been shown to give a prevalence rate of 85 % of that obtained from complete documentation, with considerable misclassification between definite and possible categories of infarction[63] Little is known about the repeatability of this kind of evaluation.

A large fraction (up to 40 %) of IHD cases die suddenly or without clinical laboratory confirmation of infarction. This fraction varies between social classes, sexes, and cultures. Classification of such deaths is important in the study of IHD because of their frequency and the fact that pathogenetic or risk mechanisms (including smoking) may be differently associated with them than with other IHD manifestations. Several considerations are involved 1) the interval between onset of acute symptoms and death, 2) the pre-existence of chronic disease, and 3) whether or not death was witnessed Sudden death may be correctly allocated to IHD in 90 % of cases in countries such as the U.S. and Sweden if the age is under 65 years and if death is witnessed to occur within 2 hours after onset of symptoms in individuals without previous chronic disease[64, 65]

6.3.1 1 Resting electrocardiogram

When distinct Q wave changes are present in the resting ECG this ECG finding is highly valid (80—95 %) for infarction by autopsy findings, and has been validated with high risk ratios for IHD death in follow-up studies[66, 67] Addition of localized repolarization abnormalities (negative T waves) as ECG criteria will increase sensitivity but

decrease specificity There is still substantial excess risk of future IHD in cases with "less than diagnostic" findings. They may be considered lower level classes related to IHD with qualified exclusions of heart diseases of other than coronary etiology and by exclusion of several well known functional disturbances and drug effects which produce similar but temporary ECG changes.

The reproducibility of ECG assessment in the hands of trained technicians, using defined criteria and standard procedure is acceptable for most study purposes and is on the order of 90 % for major ECG items[68] The disadvantages of the resting ECG are its insensitivity and distortions inherent in the records a residual of false positive and random error of classification.

The frequency of myocardial infarction in population studies should be based in part on the resting ECG taken under standardized field conditions by trained technicians mounted and coded centrally using standard criteria and trained observers, and submitted to them in such a way as to eliminate systematic error[31] Degrees of probability of infarction may be assigned by ECG and combined ECG-clinical criteria. The presence of arrhythmias, conduction defects or T wave changes may be used to characterize intermediate cases not meeting firm ECG-clinical infarction criteria but with a positive response to the Rose Questionnaire (or chest pain of half an hour duration)

6 3 1 2 ECG during exercise

The exercise ECG provides the earliest objective *method to determine myocardial ischemia* The repeatability of ECG responses to exercise is quite acceptable for practical application, with a reproducibility of the response to exercise in succeeding years ranging from 85 % to 93 %[69] In duplicate tests in patients with severe angina pectoris and angiographically verified coronary artery disease the average time difference for the appearance of S-T segment depression was 2—3 minutes on a standardized progressive test[70] Measurement agreement under standard conditions is near 90 %[68]

The principal advantage of a stress test in population studies related to IHD is the increased yield of repeatably measureable events which are relevant to IHD a yield above that obtained with other IHD measures and above that predicted by other major risk factors. *Tests at low levels of exercise* followed by a post-exercise ECG provide a 1/3 *increase in recording of the yield for both prevalence* and incidence of ischemic events over other means *for measuring IHD*[71] Tests at or near maximal work capacity *increase the yield for prevalence of* such findings by a factor of 3 based on a mean frequency of 15 % ischemic responses to exercise in the middle years in men free of overt IHD and 5 % total of overt findings from questionnaires clinical records and the resting ECG[72 73 74]

Exercise stress tests require 15 to 45 minutes, depending on complexity in a field examination schedule, and require special quarters equipment, and staff Thus they are expensive, compared to the resting ECG and other IHD survey methods. They have a finite but very small risk of morbidity or mortality (on the order of 1 serious event per 10 000 tests)

Due to the advantageous yield-cost ratio It is recommended that a standardized progressive submaximal exercise test to a fraction of the predicted maximal heart rate be considered as a study method in subsamples of the twin population. The threefold increase in frequency of reliably measurable events relevant to IHD allows an approximately 2/3 reduction in sample size required for prevalence studies Standardization of the apparatus and means for imposing the work load is not necessary at these target heart rates, but standardization is required of ECG lead systems skin electrode preparation, recording instruments recording intervals *measurement methods, and classification.*

The following precautions should be taken for applications *of exercise tests*

(1) The test should be preceded by a physician s clinical *examination and resting ECG Subjects* should be excluded from the test based on defined criteria (2) The test should permit *continuous* supervision of the subject with ECG monitoring during the entire exercise test and recovery period,

by trained technicians or a physician. (3) The test should be stopped if defined criteria of circulatory insufficiency appear prior to attaining the target heart rate a physician should be immediately available and the examining team should be trained in resuscitative procedures.

Progressive exercise to the point of producing anginal pain under controlled conditions is not recommended for routine use in volunteer subjects under field conditions because of the discomfort caused and the potential for accident.

6.3.1.3 *Other laboratory tests of ischemic heart disease*

Diagnosis of myocardial infarction in the hospital or clinic may be improved by laboratory tests such as serum transaminase measurements and sedimentation rate. Such studies obviously are limited to detailed evaluation of subgroups of large study populations that may be given a complete clinical evaluation of myocardial disease.

6.3.2 Peripheral arterial occlusive disease

The history relevant to the peripheral circulation can be determined by questionnaire[31] Sensitive methods for the study of function of the peripheral circulation allow detection of disease before symptoms of arterial insufficiency occur. These include oscillometry plethysmography blood flow measurements and temperature readings, of which oscillometry is the simplest and the most applicable to field studies. Oscillometry measurements are highly correlated to angiographic changes in the arteries. The sensitivity of the method is low for oscillations recorded over a single tissue segment or limb but increases by use of a ratio of oscillations between comparable segments on the leg and the arm (ankle and wrist) The coefficient of variation of repeated measures under standard conditions is less than 10 %

Simple recording oscillographs are available for use by trained but unskilled personnel, and a bilateral study can be performed within 10 minutes.

6.3.3 Chronic pulmonary disease

6.3.3 1 *Clinical or laboratory tests of pulmonary morbidity*

A wide variety of tests are now available and their functional implications are generally quite well understood. In terms of detection of early lung changes, in relation to the amount of equipment and personnel required to carry them out, the yield varies considerably and detection of early disease is not highly reliable. Nor is measurement of abnormalities of pulmonary function necessarily equivalent to estimating the severity of respiratory morbidity

Tests of Mechanical Properties of the Lungs These test have become the most widely applied for screening comparatively asymptomatic and ambulatory patients, for the reason that they appear to have the greatest probability of detecting early changes. In the case of bronchitis or asthma, tests related to air flow resistance will be the most affected while in early emphysema, changes in static recoil of the lungs may be present along with changes in the resistance of peripheral airways.

a. Static mechanical properties of lungs. The simplest test is the vital capacity Standards corrected for age, sex and height are available but as a screening test it is not a very sensitive indicator of early changes. With the aid of a body plethysmograph or by inert gas dilution methods measurement of residual volume and total lung capacity is possible, but such measurements have not yet been widely applied for large-scale screening. As a measure of static recoil of the lungs, deflation volume-pressure curves from full inspiration are the most informative, but esophageal pressure measurements cannot easily be done for screening purposes.

b. Dynamic mechanical properties of the lungs. Tests involving a measure of the flow resistance of the airways are now probably the most informative of all routine pulmonary function measurements. A number of indices have been extensively studied. A record of the volume expired as a function of time can be easily obtained under conditions of

maximum effort by the patient, and from such a record the vital capacity can be measured. The maximum mid-expiratory flow and peak flow can also be obtained from the tracing. The degree of cooperation of the patient required for the forced expiratory volume is not high, and consistent results can be obtained. This test has largely replaced the maximum breathing capacity because the latter requires a higher degree of cooperation. Generally the forced expiratory volume after 1 second (the $FEV_{1\cdot0}$) is measured, and expressed as a percentage of the forced vital capacity. There is some evidence now available[75] that a more sensitive test of pulmonary disturbance is the absolute value of $FEV_{1\cdot0}$. Investigators should be urged to report both measurements in absolute terms. The $FEV_{1\cdot0}$ as a percentage of forced vital capacity has less change with age than the absolute values of the separate measurements, but standards are available for comparison. In twin studies age corrections may be of less importance.

The maximum mid-expiratory flow is an excellent test, since at 50 % of the vital capacity the maximum expiratory flow is easily obtained by the patient and does not depend critically on the amount of pressure applied by the expiratory muscles. A modification of this measurement has been suggested by the analysis of maximum expiratory flow by Mead and coworkers[76] and Permutt, *et al*[77]. From a record of volume above the residual volume on the Y-axis and the maximum expiratory flow obtained by a forced expiration, on the X-axis a plot of flow against vital capacity is obtained. This is essentially a variation of the forced expiratory volume manoeuvre but the resulting plot is essentially effort independent for volumes of 70 % of vital capacity or less. Although there is not a large amount of experience accumulated with the use of this test, it appears to be one of the most sensitive for evaluating mechanical properties of the small bronchi and lung parenchyma available at this time.

Test of Uniformity of Ventilation and Perfusion of the Lung. Uniformity of both mechanical properties and the ventilation of the lungs has been extensively studied, but not under field conditions. For mechanical homogeneity study of the resistance and compliance with forced oscillations of the chest bellows can be done at different forcing frequencies. This test generally requires a body plethysmograph and gives an indication of the uniformity of time constants of regions of the lungs. Although the methods have been well worked out, and do not require training of the subject, it is unlikely that changes will be detected by this test that are not measured with the forced expiration methods mentioned above.

Dilution in the lungs by test gases such as helium, or by washout of nitrogen with the subject breathing pure oxygen[78] can measure the unevenness of the relative ventilation of different portions of the lung. Such tests require a rapid gas analyzer for measuring expired or concentration changes and thus may not be practical for field use. The sensitivity of the single breath nitrogen method has been claimed to be better than other mechanical tests by Simondsson[79] who studied a small group of patients with bronchitis under laboratory conditions. His observations should be confirmed and extended.

Measurement of gas exchange. Because ventilatory reserve is so great, tests of gas exchange at rest are no good indicators of slight degree of impairment. In addition many techniques require sampling of arterial blood, and this procedure is not suitable for large scale studies.

The diffusion capacity is not really a capacity test except during exercise when it may be a more sensitive indicator of early changes. The single breath CO uptake technique[80] or modification of it, appears to be the most reliable, but requires rapid gas analyzers for CO and a test mixing gas such as helium. This test has not had wide applicability for screening purposes, since it does not appear to be likely to detect changes in the absence of other pulmonary abnormalities.

It is possible to measure capillary blood O_2 saturation spectrophotometrically with an ear oximeter in many people, and a test proposed compares the resting saturation with saturation with the subject breathing pure oxygen. The equipment is relatively simple to operate, but there is no

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MEDICALLY UNATTENDED FATAL CASES OF ISCHAEMIC HEART DISEASE IN A DEFINED POPULATION

Incidence during one year in Stockholm, with particular reference to prevalence of certain previously diagnosed disorders, some characteristics of the last attack, and postmortem findings

By Bo Wikland

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MEDICALLY UNATTENDED FATAL CASES OF ISCHAEMIC HEART DISEASE IN A DEFINED POPULATION

From the Department of Medicine
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Medically Unattended Fatal Cases of Ischaemic Heart Disease in a Defined Population

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By

BO WIKLAND

STOCKHOLM 1971

Translated from the Swedish

by

JOHN HOGG

Tryckeri Balder AB Stockholm 1971

If clinical research is to be used to get the full picture of disease it must equip itself to carry observations beyond the hospitals

SIR JAMES SPENCE

Translated from the Swedish

by

JOHN HOGG

Tryckeri Balder AB, Stockholm 1971

Contents

Preface

Viewed in the light of the advances of recent years in the results of treatment for complications of acute manifestations of ischaemic heart disease (IHD) especially the early potentially serious arrhythmias, the fatal cases of this disease occurring outside hospital have appeared as a frustrating obstacle to the optimal application of these principles of treatment.

In 1967 Professor Gunnar Biörck, Head of the Department of Medicine, Karolinska Institutet at Serafimerlasarettet, brought up the idea of charting the medically unattended deaths from IHD in Stockholm, and the present survey is a direct continuation of an earlier investigation on these lines. I am greatly indebted to him for valuable advice and points of view as well as for placing the necessary facilities at my disposal. Knut Westlund, M.D. Oslo, consultant medical statistician generously contributed his outstanding experience to the design of the study analysis of the results and review of the manuscript. Miss Anne-Marie Bolander statistician of the National Central Bureau of Statistics, introduced me to the principles of statistics relating to causes of death. I have followed her advice especially in the elaboration of the first part of the study. Torbjörn Lundman, M.D. and Erik Orinius, M. D. reviewed the manuscript, and both provided valuable and constructive criticism of the presentation of the results. I am also indebted to the former for introduction to computer analysis. Close collaboration with the pathologists, headed by Sven-Olof Lidholm, M.D. at the Government Institute for Forensic Medicine was necessary throughout the study. Their contribution is gratefully acknowledged. Excellent secretarial assistance in the preparation of the manuscript was offered by Mrs Vreca Hultén.

These are only a few of the many persons who contributed to the fulfilment of this study. To all who assisted in various capacities I extend my warm thanks.

The study was supported by grants from the Swedish National Association against Heart and Chest Diseases, the Folksam Insurance Company and from Karolinska Institutet for computer analysis.

Introduction

Fatal cases attributed to ischaemic heart disease (IHD) occurring outside hospital largely failed to attract the interest of clinicians as long as the hospital mortality rate for this disease remained virtually unaffected. However during the last decade we have witnessed a real breakthrough in the treatment of complications associated with acute manifestations of IHD particularly in the field of arrhythmias not secondary to symptoms of severe heart failure. These primary arrhythmias are most likely to occur shortly after the onset of an acute attack. The most spectacular result of such progress is the introduction of the coronary care unit (CCU) in which are concentrated facilities for rapid detection and treatment of arrhythmias complicating an acute attack of IHD. The results reported (Killip & Kimball, 1967 Lawrie *et al* 1967 MacMillan *et al* 1967 Oliver *et al* 1967 Restieaux *et al* 1967 Lindman *et al* 1969 Hofvendahl, 1971) from centres with CCU are optimistic in terms of reduced mortality rates for acute myocardial infarction during the period of hospitalization. In addition, although experience is still limited, the long term prognosis for cases with successfully treated arrhythmia, which probably would have been fatal outside the CCU is reported to be comparable to that for cases with uncomplicated myocardial infarction (Geddes *et al* 1967 Lawrie, 1969 Stannard & Sloman, 1969 McNamee *et al* 1970)

The CCU is now considered an established institution, and in Sweden CCUs are being set up at an increasing number of hospitals. This gives rise to the question to what extent could the total mortality attributed to IHD be expected to be reduced in a community optimally provided with hospital CCU facilities? According to Lown *et al.* (1969) the possible additional reduction of mortality under these conditions would be no more than 2—3 per cent. This forecast was based on the experience of several community studies (Elsenberg *et al* 1961 Spiekerman *et al* 1962 Bainton & Peterson, 1963 Kannel *et al* 1963 Mathewson *et al* 1965 Kuller *et al* 1966 Armstrong, 1968 Dewar & Floyd, 1968 Fry 1968 McNeilly & Pemberton, 1968 McWinney 1968) reporting that the majority of the deaths attributed to IHD occurred outside hospital. One explanation for this distribution of deaths is offered by the uniform findings of the community studies on IHD in Belfast (McNeilly & Pemberton, 1968) and Edinburgh (Armstrong, 1968) dealing with the time relationship between the onset of symptoms of the last attack and death. From these studies it was reported that one third to one half of all fatal cases occurred within an hour of onset of symptoms of the critical attack.

As it is a truism that the patho-physiological mechanisms relating to medically unattended death ascribed to IHD remain concealed for the foreseeable future, it would seem a fair guess, on the basis of available clinical and postmortem evidence, that in most cases the ultimately fatal mechanism was kindred to primary arrhythmia. CCU experience suggests that, on admission the patients subsequently developing potentially hazardous arrhythmias do not generally present with any specifically alarming signs or symptoms heralding this particular complication Also, these patients are generally unaware of disorders of the heart rhythm presaging life-threatening primary arrhythmia observed on electrocardiographic monitoring. On the other hand, though patients developing signs of severe heart failure due to extensive infarction usually deteriorate less rapidly serious arrhythmias secondary to these symptoms carry a poor

prognosis even under CCU conditions. Thus CCU experience applied to the sudden and medically unattended deaths attributed to IHD seems to suggest that primary arrhythmia is the ultimately lethal mechanism in the majority of cases

Postmortem examinations in cases of sudden and unexpected death attributed to IHD usually do not provide entirely satisfactory findings in explanation of the fatal outcome In three U.S Medical Examiner series (Weinberg & Helpern, 1959 Spain *et al* 1960 Adelson, 1961) comprising almost 1400 cases attributed to this disease, atherosclerotic coronary artery lesions only were found in 69 per cent of the cases, whereas thrombotic occlusions were noted in 31 per cent. In a series of fatal, medically unattended cases attributed to IHD in Stockholm, which had been referred to forensic postmortem examination, lesions obviously incompatible with life, such as myocardial rupture with cardiac tamponade, were found in less than 5 per cent (Wikland, 1968) Accordingly in most cases of medically unattended death attributed to IHD postmortem examination does not contradict the hypothesis of primary cardiac arrhythmia as the ultimate cause of the fatal event.

To sum up the situation to-day hosp tal CCUs have doubtless proved that otherwise probably fatal arrhythmia complicating the acute manifestations of IHD can be effectively prevented and treated However mainly as a consequence of the highly unfavourable time relationship between the onset of an acute attack and death, most deaths occur before CCU facilities can be made fully available. In addition, circumstantial evidence suggests that the majority of the medically unattended deaths attributed to IHD are the result of potentially reversible arrhythmias which per se, f successfully treated do not appear to affect the long term prognosis adversely Consequently the a ailability of effective means to reduce the mortality due to primary arrhythmia associated with an acute attack of IHD has created a situation characterised as perhaps the greatest therapeutic challenge of the moment (Fulton *et al* 1969)

In the case of IHD as long as prevention of the underlying morb d condition emains at a tentative stage, no ideal strategy against the potentially reversible fatal attacks attributed to this disease can be conceived For the moment it appears that measures to shorten the critical period from onset of an acute attack to access to full CCU facilities represent the only practicable means by which the early death toll of IHD could be reduced

A tactical approach to reduce the delay between the onset of an acute attack and the initiation of coronary care, and to minimize the hazards of transportation, has been proposed by several authors (Moiseev 1962 Pantridge & Geddes 1967 Chasov 1968 Dewar & Floyd 1968 Kernohan & Gucken, 1968 Adgey *et al* 1969 Dewar *et al* 1969 Grace & Chadbourn, 1969 Barber *et al* 1970) using a mobile CCU The results presented hitherto (Pantridge & Geddes, 1967 Dewar & Floyd, 1968 Kernohan & Gucken 1968 Adgey *et al* 1969 Dewar *et al* 1969 Grace & Chadbourn, 1969 Barber *et al* 1970) suggest that the mobile CCU is a practicable proposition but the results, however encouraging, do not permit an assessment of its future role in consideration of the problems involved The staff of the mobile CCU must be well trained at a hospital CCU and they must remain at a high degree of alertness all round the clock. As pointed out by the Belfast mobile CCU team (Adgey & Zaidi 1969) who have gained the greatest experience in this field, the results largely depend on close cooperation with family doctors and other physicians working outside hospital and, ultimately with the public. From the Edinburgh study (Oliver 1968) it was pointed out *that the biggest delay before coronary care could* be obtained was represented by the time taken before a patient called his doctor

In Stockholm no measures had been taken at the start of this survey to reduce the mortality from IHD outside hospital. In fact, as far as the Stockholm area was concerned it was not known how large a proportion of the total IHD mortality among the population occurs outside hospital From an earlier study (Wikland, 1968) it was known that the Stockholm area does offer satisfactory prospects for the study of deaths outside hosp tal. A large number of these cases are autop-

sied after routine investigation by the police, even when the investigation has not given rise to a suspicion of violent cause.

Aims of the survey

In the light of this situation the present survey was initiated with the following aims

(1) To record the total mortality from IHD among a defined population representing the Stockholm area during a period of one year

(2) To group these cases into the following categories medically unattended deaths, hospital deaths and deaths in other institutions for the chronically ill or aged

(3) To study the medically unattended deaths from the following aspects duration of last attack and activity at onset of symptoms of this attack, attempts made during the attack to call for medical assistance, prevalence of a history of certain pre ious disorders, and autopsy findings.

(4) To study the medically unattended deaths attributed to non-violent causes other than IHD occurring within the same area and during the same period as in the study of correspond ng fatal cases of IHD and autopsied at the same institution as the medically unattended cases attributed to IHD The object of investigating these cases, apart from obtaining an idea of the size of this group in relation to corresponding fatal cases of IHD was to get a picture of (a) the spectrum of causes to which these cases were *attributed* (b) the prevalence of a history of the following disorders previous myocardial infarction, angina pectoris, heart failure, hypertension, and diabetes and (c) the prevalence of lesions attributable to IHD found at autopsy

(5) Finally it was hoped that on the basis of the findings in the present study some clues could be derived as to possible approaches to reduce the share of the death toll of IHD represented by medically unattended cases.

Overall mortality from ischaemic heart disease

MATERIAL, METHODS, AND DEFINITIONS

The period studied extended from July 1 1968 to June 30 1969 The risk population consisted of the registered population of Stockholm and of the following communes Boo, Danderyd, Djursholm, Huddinge, Lidingö Nacka, Saltsjöbaden, Sollentuna, Solna, Sundbyberg, Tyresö and Täby

The cases studied consisted of all deaths from ischaemic heart disease (IHD) in this population during the year of study

The National Central Bureau of Statistics (NCBS) Stockholm, keeps the medical certificates of causes of death for the following cases

a. all deaths occurring in Sweden,

b deaths occurring abroad of persons registered in Sweden

A perusal was made of the death certificates for all deaths occurring in the det ned risk population the period of study From the certificates all cases of death from IHD were selected on the basis of the following criteria.

When entered anywhere on Part I of the medical certificate of the cause of death the following diagnoses qualified for entry in the study

Acute myocardial infarction (ICD* 410) other acute and sub-acute forms of IHD (ICD 411) chronic IHD (ICD 412) and angina pectoris (ICD 413)

Age at death s given as the age attained at the last birthday

On the basis of particulars in the death certificates the cases were classified in four categories. When the death certificate did not contain sufficient data for such classification, the physician issuing the death certificate was asked for supplementary information The four categories were as follows

1 *Medically unattended deaths* To this group were assigned deaths outside hospital or other institution for the chronically ill or aged.

2. *Hospital deaths* To this category were assigned deaths in the following hospitals Danderyd, Ersta, Karolinska, Löwenströmska, Nacka, Roslagstull, Sabbatsberg, St Erik, St Göran, Serafimerlasarettet, Södersjukhuset, and Södertälje hospital

Persons who belonged to the risk population at the time of death and who died in a hospital for emergency admissions outside the Stockholm area were also included in this category

3 *Deaths in other institutions for the chronically ill or aged* This category includes cases occurring in mental hospitals, convalescent homes, homes for the aged, and other institutions for the chronically ill or aged, with the exception of the previously listed hospitals. This category is hereinafter referred to as deaths in other institutions

4. *Deaths abroad* The available information in these cases was altogether too inadequate to allow classification in any of the three aforesaid categories These cases, however represented only a very small fraction (0 7 per cent) of the total number of deaths in the survey A separate calculation of death rates for this group was not made, therefore, but the cases were included in the calculations of the total death rates.

Mean population data were obtained from official population statistics (Stockholm Office of Statistics, 1969) for the end of the calendar year 1968 i.e the middle of the period studied.

At the NCBS the causes of death were coded according to the 7th revision of ICD until the end of 1968 From 1969 and onwards the 8th revision

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* International Classification of Diseases, Detailed List Nos 8th revision 1965

was applied. To obtain uniformity for the whole period, the deaths of 1968 were reclassified according to the 8th revision on the basis of a comparative study of the 7th and 8th revisions carried out by the NCBS. Although unpublished, the results of this study were placed at the author's disposal. According to this study the ICD Detailed List codes of the 8th revision, defined as IHD deaths in the present study that is to say Nos 410, 411 412, and 413 were found to correspond to the following codes of the 7th revision 420.0 420 1 420.2 and 422 1 The death rates for IHD as defined above were then computed for Sweden for the period 1966—1968 on the basis of Official Statistics of Sweden (Causes of Death 1966 1967 and 1968)

Statistical methods

Unless otherwise indicated, mean age was calculated from the arithmetic mean of five-year age class means. Conventional methods were used for the calculation of the arithmetic mean and the significance of difference between mean values was tested by Student's t-test. The chi-square test was used for testing the significance of differences of relative numbers. Yates correction was applied when small numbers were employed. The significance of linear trends in proportions and frequencies was tested according to the formula suggested by Armitage (1955) Degrees of significance were tested at the 5 1 and 0 1 per cent levels.

A difference found to be significant at a probability level above 5 per cent was considered non-significant When between the 5 and 1 per cent levels of probability the significance of a demonstrated difference was considered to be almost significant Significance levels under 1 per cent were considered significant

RESULTS

A total f 3304 deaths from IHD occurred. The age and sex distributions of the cases are shown in table 1 and in figures 1 and 2. In the same table and in figures 3 and 4 are also given the computed annual IHD death rates per 100 000 mean population by sex and five-year age groups, both for the population studied and for Sweden

TABLE 1 IHD deaths (see text for definition) and annual death rates by sex and age Present study and Sweden 1966—68

	Present study				
				Revised[1] rates	Sweden 1966—68
Age	No.	Per cent	Per 100,000	Per 100,000	Per 100,000
		MALES			
30—34	2	1 2	6 2	6.2	1.8
35—39	6		19.5	13 0	11 2
40—44	13		37 4	34.5	34.5
45—49	38	2.2	92 4	92.4	83 6
50—54	58	3 3	159 4	156.7	169 4
55—59	111	6 4	311 4	294.6	331 4
60—64	206	11 8	711 4	694 1	624 4
65—69	265	15.2	1261 3	1204 2	1089 7
70—74	308	17 7	2106 6	2079.2	1795.3
75—79	318	18 3	3404 0	3232 7	2820 8
80—84	247	14.2	5012.2	4849.8	4433.8
85—	168	9 7	7887 3	7632 6	7395 2
Total	1740	100 0			
		FEMALES			
30—34	1	4	3 2	3.2	1 1
35—39	—		—	—	1 9
40—44	1		2.7	—	5 5
45—49	5		11.5	11.5	15 5
50—54	15	1.0	37.2	34 7	33 6
55—59	29	1 9	69 7	67.3	76.1
60—64	74	4.7	198 7	196.1	190 5
65—69	135	8 6	445 1	428.6	437 9
70—74	219	14 0	923.2	876 8	926 9
75—79	304	19 4	1740 0	1665 6	1873 0
80—84	377	24 1	3661 3	3486.5	3557 5
85—	404	25 8	7591 1	7159 0	6652 0
Total	1564	100 0			

[1] According to WHO rules for selection of underlying cause of death

1966—1968 The former rates are also given in revised form in order to increase comparability with the national rates

Of the 3304 cases of IHD death 1740 (53 per cent) were male and 1564 (47 per cent) female. The absolute and relative age distributions of the various categories of male deaths are shown in table 2 and in figure 1 The corresponding distributions of female deaths are shown in table 2 and in figure 2. The death rates for males in the various categories are shown in table 3 and in figure 3 the

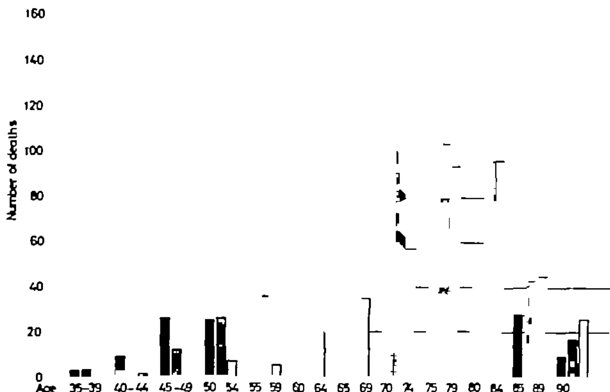


Figure 1 IHD deaths (see text for definition) by category and age. ■ medically unattended deaths· □ hospital deaths· □ deaths in other institutions.

corresponding rates for females are shown in the same table and in figure 4

As appears from the totals in table 2, 39 3 per cent of the male deaths were medically unattended, 38.4 per cent occurred in hospital, and 21 4 per cent in other institutions The corresponding figures for females, as appears from table 2, show a very different distribution, 21 7 per cent of the female deaths being medically unattended, 36.6 per cent occurring in hospital, and 41.2 per cent other institutions

The mean age at death for males was 68 3 years for medically unattended cases and 71 4 years for hospital cases The d fference is statistically significant ($p<0\ 001$) Likew se the mean age for male deaths n hospital 1 4 years, differs significantly ($p<0\ 001$) f om that for male deaths in other institutions, which was 77 6 years

The mean age at death for medically unattended females, 73.2 years, differs significantly from that recorded for hospital cases, 76.3 years ($p<0.001$) Likewise the mean age at death of hospital cases 76.3 years, is significantly lower than that for cases in other institutions, 82.7 years ($p<0.001$) The mean ages at death for medically unattended cases compared with those occurring in other institutions also showed significant differences for both sexes ($p<0.001$) A more detailed account of the age distributions will be found for each sex in table 2.

According to the death certificates the diagnosis in most cases in the present survey was based on the postmortem examination. Of the deaths occurring outside hospital 78 per cent had been autopsied according to the death certificates. The corresponding figure for hospital cases was 94 per cent and for other institutions 62 per cent.

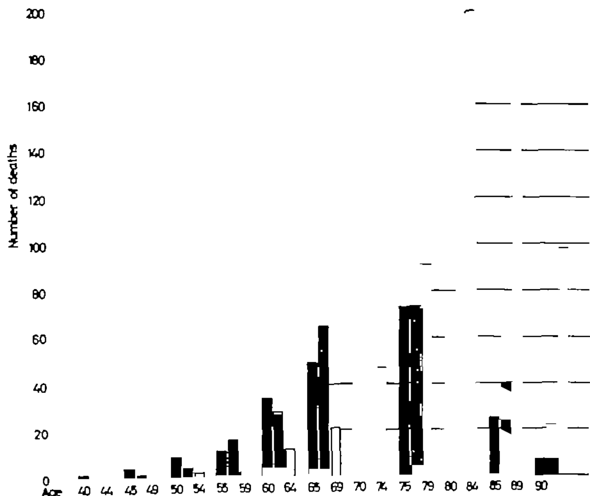


Figure 2 IHD deaths (see text for definition) by category and age ■ medically unattended deaths ■ hospital deaths; □ deaths in other institutions

COMMENTS

For primary tabulations of causes of death only the "underlying cause" selected according to WHO rules for classification, is regarded in official statistics. When revised according to these rules, as will be seen in table 1 the death rates of the present series become slightly lower than those obtained by the criteria used in this survey Still, when compared with the IHD death rates for Sweden 1966—1968 the revised rates of the present series remain somewhat higher The remaining differences, however entirely coincide with the ones to be expected between the national rates and those of the Stockholm area, according to the results of a study carried out at the NCBS (1971)

According to experience from several British and American studies of total IHD mortality the majority of deaths occur outside hospital (Eisenberg *et al* 1961 Spiekerman *et al* 1962 Bainton & Peterson, 1963 Kannel *et al* 1963 Mathewson *et al* 1965 Kuller *et al* 1966 Armstrong, 1968 Dewar & Floyd, 1968 Fry 1968 McNeilly &

TABLE 2 IHD deaths (see text for definition) by sex age and category

Age	Medically unattended N	Medically unattended Per cent	Hospital N	Hospital Per cent	Other institutions No.	Other institutions Per cent	Abroad No	Total No	Total Per cent
				MALES					
30—34	1		1		—		—	2	
35—39	3	1 9	3	1.0	—	.3	—	6	1.2
40—44	9		3		1		—	13	
45—49	26	3 8	12	1.8	—		—	38	2.2
50—54	25	3 7	26	3 9	4	1 1	3	58	3 3
55—59	70	10 2	36	5 4	3	.8	2	111	6.4
60—64	102	14 9	83	1[illegible]4	20	5 4	1	206	11.8
65—69	128	18 7	102	15 3	33	8.8	2	265	15.2
70—74	112	16.4	139	[illegible]0.8	55	14 8	2	308	17 7
75—79	120	17 5	104	15.6	92	24.7	2	318	18 3
80—84	51	7 5	99	14 8	95	25 5	2	247	14.2
85—89	28	4.1	43	6.4	44	11.8	1	116	6 7
90—	9	1 3	17	2.5	26	7 0	—	52	3 0
Total	684	100 0	668	100 0	373	100 0	15	1740	100 0
Per cent of all males	39 3		38 4		21 4		.9	100.0	
Mean age (years)	68 3		71.4		77 6		67 7	71.5	
				FEMALES					
30—34	—		1		—		—	1	
35—39	—	1 5	—	3	—		—	—	4
40—44	1		—		—	.6	—	1	
45—49	4		1		—		—	5	
50—54	9	2.7	4	7	2		—	15	1 0
55—59	11	3.2	16	2.8	2		—	29	1.9
60—64	34	10 0	28	4 9	10	1.6	2	74	4.7
65—69	49	14.5	65	11 4	20	3 1	1	135	8 6
70—74	67	19.8	105	18 4	46	7 1	1	219	14 0
75—79	73	21.5	140	24 5	91	14 1	—	304	19 4
80—84	59	17 4	117	20.5	200	31.0	1	377	24 1
85—89	25	7 4	73	12.8	176	27 3	3	277	17 7
90—	7	2.1	22	3 9	98	15.2	—	127	8.1
Total	339	100 0	572	100 0	645	100 0	8	1564	100 0
Per cent of all females	21 7		36.6		41.2		5	100 0	
Mean age (years)	73.2		76 3		82 7		75 8	78 5	

Pemberton, 1968 McWinney 1968) A Danish study (Mosbech & Dreyer 1965) reported that, of all IHD deaths in the country 44 per cent occurred in departments of internal medicine in large hospitals or in undepartmentalized smaller provincial hospitals. In none of the cited studies was there a report of deaths in the type of institution here referred to as "other institutions for the chronically ill or aged In a statement concerning the situation in Belfast at the time of the study by McNeilly & Pemberton (1968) Pemberton said (1969) that in Belfast there are no special hospitals for the chronic sick or aged people, but that wards or a block of wards in a large city hosp tal are devoted

TABLE 3 IHD deaths per 100,000 per year by sex, age and category

Age	Medically unattended	Hospital	Other institutions	Total (including abroad)	Sweden 1966—68
			MALES		
30—34	3.1	3.1	—	6.2	1.8
35—39	9.8	9.8	—	19.5	11.2
40—44	25.9	8.6	2.9	37.4	34.3
45—49	63.2	29.2	—	92.4	83.6
50—54	68.8	71.5	11.0	159.4	169.4
55—59	196.7	101.2	8.4	311.4	331.4
60—64	351.9	286.4	69.0	711.4	624.4
65—69	609.3	485.5	157.1	1261.3	1089.7
70—74	766.1	930.8	376.2	2106.6	1795.3
75—79	1284.0	1112.8	984.4	3404.0	2820.8
80—84	1034.8	2008.7	1927.6	5012.2	4433.8
85—	1737.2	2817.0	3286.5	7887.3	7395.2
			FEMALES		
30—34	—	3.2	—	3.2	1.1
35—39	—	—	—	—	1.9
40—44	2.7	—	—	2.7	5.5
45—49	9.2	2.3	—	11.5	13.5
50—54	22.3	9.9	5.0	37.2	33.6
55—59	26.5	38.6	4.8	69.7	76.1
60—64	91.5	75.3	26.9	198.7	190.5
65—69	161.7	214.5	66.0	445.1	437.9
70—74	282.7	443.1	194.1	923.2	926.9
75—79	417.6	800.8	520.5	1740.0	1873.0
80—84	572.9	1136.1	1942.0	3661.3	3357.5
85—	601.3	1785.1	5148.5	7591.1	6652.0

to this category of case. In the Belfast study it was found that 60 per cent of all IHD deaths occurred before the patients reached hospital and that 30 per cent of the patients died after reaching hospital. The remaining 10 per cent died in hospital but had been admitted to hospital for other than cardiac conditions. According to Pemberton (1969) the majority of the latter group consisted of geriatric cases. In the Danish study Mosbech & Dreyer (1965) found the mean age for men dying from IHD in hospital to be 68.0 years compared with 69.9 years for men dying outside hospital. The corresponding ages for women were 72.4 and 74.4 years respectively.

In a Swedish study (Fodor, 1969) relating to Gothenburg it was found that, of all IHD deaths among the population during a 3-month period, 37 per cent occurred outside hospital. Up to 65 years of age 57 per cent of the deaths occurred outside hospital, and above 65 years of age 31 per cent. In the present study deaths outside hospital totalled 31 per cent, the proportion of deaths outside hospital below 65 years of age 53 per cent, and from 65 years and upwards 27 per cent. In the cited study from Gothenburg the deaths in hospital included patients dying in departments of internal medicine, surgery, infectious diseases, neurology and gynaecology, as well as in psychiatric and geriatric departments.

In the present study, apart from deaths at Serafimerlasarettet, no analysis was made of the breakdown of deaths in hospital by departments in which they occurred. Of the 139 deaths of IHD at Serafimerlasarettet 129 (93 per cent) occurred in the Department of Medicine (including CCU). The remaining 10 cases (7 per cent) occurred in the

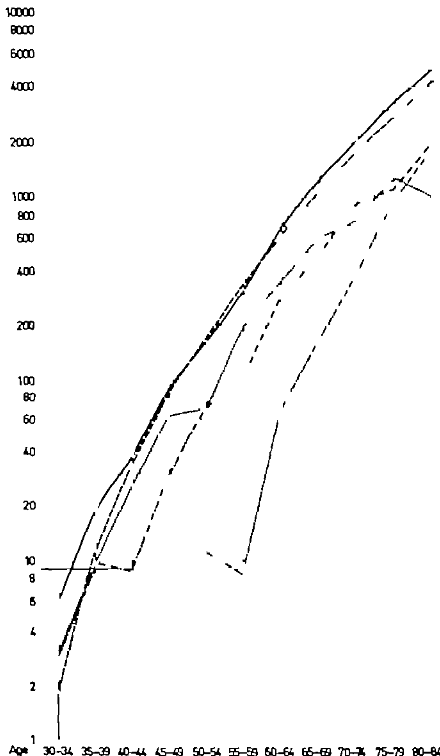


Figure 3 IHD deaths (see text for definition) per 100,000 per year by category —— total present series —— Sweden 1966—68 —— medically unattended deaths ---- hospital deaths —— deaths in other institutions

FEMALES

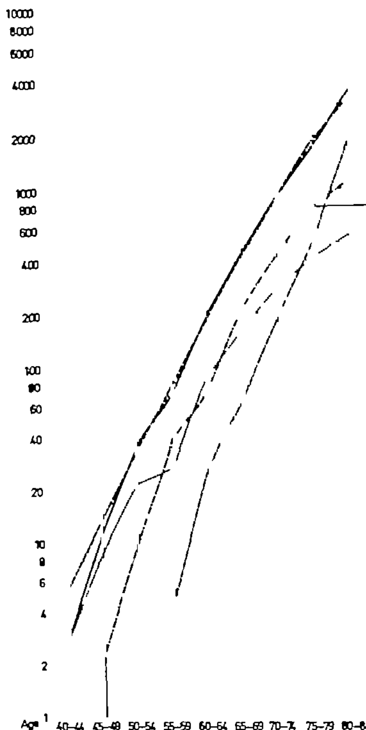


Figure 4 IHD deaths (see text for definition) per 100,000 per year by category ——— total present series ——— Sweden 1966—68 ——— medically unattended deaths —·— – hospital deaths ——— deaths in other institutions

Department of Surgery It appears reasonable, in the light of the results from Serafimerlasarettet, to assume that about 10 per cent of IHD deaths in the remaining hospitals, which have wards for other specialties, occurred in a department other than that to which IHD was the cause of admission.

SUMMARY

A record was made of all deaths in IHD occurring during one year among the population in Stockholm and neighbouring communes (a total population of nearly 1 1 million)

Of the 3304 deaths, 1740 (53 per cent) occurred among men and 1564 (47 per cent) among women. The deaths were classified in the following categories 1 Medically unattended deaths, 2 Hospital deaths and 3 Deaths in other institutions for the chronically ill or aged In the order mentioned the numbers of male deaths were 684 (39 3 per cent) 668 (38.4 per cent) and 373 (21 4 per cent) and of female deaths 339 (21 7 per cent) 572 (36.6 per cent) and 645 (41.2 per cent)

The mean age at death in the various categories was for males 68 3 years for medically unattended deaths, 71 4 years for hospital deaths and 77 6 years for deaths in other institutions, and for females 73.2, 76 3 and 82 7 years, respectively

The overall mean age for men was 71 5 years and for women 78.3 years

Medically unattended deaths attributed to ischaemic heart disease

MATERIAL AND METHODS

Basic assumptions for the study

All deaths occurring outside hospital or other institutions for chronically sick or aged people in the area must be reported to the police. With few exceptions such cases are taken to the Government Institute for Forensic Medicine, Karolinska Institutet, which also functions as central morgue for the area. After investigation the police decide which cases shall be autopsied. Apart from cases judged by the police to have been caused or possibly caused by violence, a postmortem is made also in some cases when there is no suspicion of crime. In cases when there is no suspicion of crime the police investigates whether the deceased was undergoing medical treatment. If the police can trace the deceased's physician, the latter is asked whether, from his knowledge of the patient's medical history he is prepared to issue a death certificate. If so a postmortem is not made. But if the physician does not consider he can issue a death certificate, or if the deceased's physician cannot be traced, a postmortem is made at the Government Institute for Forensic Medicine.

Sources of information

In cases autopsied at the Government Institute for Forensic Medicine there is always a copy of the police report as basis for the medical examiner's judgment of the extent of examination necessary. From the most detailed police reports the following data were obtained witness description of the circumstances surrounding the death, particulars of the deceased's earlier state of health, dates of treatment in hospital, and name and address of the patient's physician. The detailed reports also contained statements of the police's own observations on the spot, with a reconstruction of the situation in which the death occurred, medicines found, and name of the prescribing physician or physicians. Particulars were often given of the quantity of medicines consumed. From the most summary reports data were obtained in cases when the death was witnessed—or the person was found dead—in the form of a brief summary of the witness account of the circumstances surrounding the death and the necessary observations made by the police to exclude the possibility of a violent cause.

Supplementary information was obtained from hospital records, from the physicians concerned, from the Social Insurance Office, and from relatives. A perusal was also made of the death certificates at the National Central Bureau of Statistics (NCBS) so as to include cases not recorded at the Government Institute for Forensic Medicine.

The validity of diagnosis will be discussed later in section F. Primarily the validity of the diagnosis was considered to differ for the autopsied and non autopsied cases. The material was therefore divided into autopsied and non-autopsied cases.

Composition of the material

The deaths studied in this part of the survey (Part II) are those which occurred within an area corresponding to the Stockholm telephone area during the period July 1 1968—June 30 1969 and which, in accordance with the criteria adopted in the survey of overall IHD mortality (Part I) were medically unattended and had been caused by IHD. From the map (appended) will be seen the boundaries of the studied area in relation to the boundaries of the communes* of which the population was studied in respect of overall IHD mortality in Part I.

Shown in the map as solid line

Shown in the map as broken lines.

TABLE 4 Medically unattended deaths The final series by sex, age and autopsy status

	MALES					FEMALES					
	Autopsied		Non-autopsied		All males	Autopsied		Non-autopsied		All females	Total
Age	No.	Per cent	No.	Per cent	N	No.	Per cent	No	Per cent	No	No.
30—34	1	2.1	—	—	1	—	—	—	—	—	1
35—39	3		—	—	3	—	—	—	—	—	3
40—44	7		—	—	7	1	4	—	—	1	8
45—49	22	4.2	1	9	23	4	1.6	—	—	4	27
50—54	25	4.8	3	2.6	28	7	2.7	2	2.6	9	37
55—59	51	9.8	10	8.6	61	9	3.5	1	1.3	10	71
60—64	84	16.2	13	11.2	97	32	12.5	2	2.6	34	131
65—69	90	17.3	28	24.1	118	38	14.8	9	11.8	47	165
70—74	83	16.0	20	17.2	103	54	21.1	11	14.5	65	168
75—79	92	17.7	17	14.7	109	58	22.7	16	21.1	74	183
80—84	38	7.3	13	11.2	51	40	15.6	19	25.0	59	110
85—89	16	3.1	9	7.8	25	10	3.9	12	15.8	22	47
90—	7	1.3	2	1.7	9	3	1.2	4	5.3	7	16
Total	519	100.0	116	100.0	635	256	100.0	76	100.0	332	967
Mean age (years)	67.7		71.1		68.3	72.0		77.4		73.2	

As appeared in Part I, 1023 of the IHD deaths were medically unattended, 121 of which occurred outside the Stockholm telephone area. On the other hand 65 medically unattended deaths occurred among persons who though staying in the area, had not been included because they were not registered in any of the parishes within the communes studied in Part I. Therefore 967 cases were available for study

The distribution by sex, age, and autopsy status of the cases dealt with in the present part of the study is shown in table 4. As will be seen, the mean age for the autopsied and non autopsied cases, both among men (68.3 years) and women (73.2 years) in this series was similar to the corresponding mean ages for the medically unattended deaths in the population reported in Part I (table 2)

As appears from table 4, a postmortem had been made in 519 (82 per cent) of the 635 male cases and in 56 (77 per cent) of the 332 female cases The tabl also shows that the mean age for the autopsied cases was lower than for the non-autopsied cases of the same sex (for males $p<0.01$ and for females $p<0.001$)

COMMENTS

A similar procedure to that described in cases of medically unattended death in Stockholm is followed also in Gothenburg and Malmö second and third largest of Swedish cities. The high autopsy rate in such cases appears to be unique by inter national standards From the USA Myerburg & Davis (1964) reported that the sudden deaths of IHD certified by the Medical Examiner constituted about 7—9 per cent of all deaths due to IHD among persons under the age of 65 However far from all cases referred to the Medical Examiner are autopsied Spain *et al* (1960) reported that, of their cases of natural death referred to the Medical Examiner half were autopsied From the study of McNeilly & Pemberton (1968) including all fatal cases of IHD in Belfast, an overall autopsy rate of 30 per cent was reported. Autopsy rates comparable with that reported in the latter study were obtained for other Swedish areas than Stock holm, Gothenburg and Malmö. Thus, according to information provided in the death certificates kept at the NCBS, in 1961—1967 the cause of death when assigned to arteriosclerotic and degenerative

heart disease (ICD A 81 Detailed List Nos. 420—422, 7th revision) in this population had been established by autopsy for approximately 30 per cent of both sexes.

It may seem unwarranted in this part of the study to adopt other criteria for recording of medically unattended deaths than those applied to the population survey in Part I, namely to include the deaths occurring in the Stockholm telephone area. This change of the qualifications for entry in the present part of the study was, however due to several reasons One of the questions which initiated this part of the study was whether the establishment of an ambulance service available to the public, of mobile CCU type, might be justified in the Stockholm area. It appeared necessary for this purpose to assume that phone calls from the public for this ambulance would be limited to cases occurring within an area which, at least theoretically could be meaningfully served from the central parts of Stockholm—that is to say with the aim of shortening the delay before making the means of cardiac resuscitation fully available. By using the Stockholm telephone area as boundary for the area studied, it was also easy to check where the death occurred and thereby moreover to gain a better picture of the actual situation in that area.

The fact that the mean age for the non-autopsied cases of both sexes was higher than for the autopsied must be viewed in the light of the principles for the issue of a death certificate without preceding postmortem. It appears reasonable to presume that the non-autopsied cases included some of the medically unattended deaths in which, to a greater extent than in the autopsied cases, the deceased persons had had time to develop a symptomatic manifestation of IHD or of a disorder commonly associated with IHD resulting in contact with a physician. These aspects will be touched upon in conjunction with the review of the prevalence of certain previous disorders.

A. DURATION OF LAST ATTACK

As shown from Belfast (McNeilly & Pemberton, 1968) and Edinburgh (Fulton *et al* 1969) among other places, an important reason why many persons dying of IHD do not come under an adequate form of care is the very unfavourable time relation between the onset of symptoms of the critical event and death. As, in the consideration of conceivable preventive measures this time relation must be of fundamental significance, an attempt was made in this material as well to delimit in time the acute symptoms during the fatal episode.

Basis for evaluation of the duration of the last attack

Witnesses statements were required for the duration of the acute symptoms in conjunction with the fatal event to be considered known. These statements were obtained to a large extent from the police reports. When the information provided by the police reports was insufficient or—as in non autopsied cases—a copy of the police report was not available relatives or the physician who issued the medical certificate of the cause of death were questioned concerning the time interval between the onset of symptoms and death.

The symptoms attributed to the last attack were chest pain, with or without radiation from the chest, and dyspnoea. In most cases in which the duration of attack was less than one minute, no report of such symptoms could be obtained. At the other extreme there were cases with lingering symptoms and insidious onset of chest pain or dyspnoea for days or sometimes weeks prior to death. In such cases the symptoms of the last attack were considered to have started at the time from which a statement existed concerning continuous symptoms in the form of chest pain or dyspnoea preceding death. As it was often difficult to estimate the duration of the fatal episode when it exceeded two hours these cases were placed in the same group.

RESULTS

The duration of the symptoms attributable to the fatal event is shown by sex and autopsy status in table 5 It is seen from this table that approximately half of the cases with known duration of symptoms referable to the last attack died within 15 minutes of the onset of these symptoms. In a comparison between the sexes however, there were certain differences as regards the duration of the acute symptoms Thus as appears from table 5

TABLE 5 Medically unattended deaths by sex, duration of last attack and autopsy status

		Minutes						All know	Un-known	Total
		0—1	2—15	16—30	31—60	61—120	121—			
Autopsied										
Males	No.	141	65	23	17	15	85	346	173	519
	Per cent	41	19	7	5	4	25	100		
Females	No.	47	18	12	9	11	40	137	119	256
	Per cent	34	13	9	7	8	29	100		
Non-autopsied										
Males	No.	34	13	5	5	3	11	71	45	116
	Per cent	48	18	7	7	4	16	100		
Females	No.	13		—	4	3	14	41	35	76
	Per cent[1]	32	17	—	10	7	34	100		
Total	No.	235	103	40	35	32	150	593	372	967
	Per cent	40	1	7	6	5	25	100		

Of cases with known duration of last attack

of the autopsied males with known duration of attack, 206 (60 per cent) died within 15 minutes compared with 65 (47 per cent) of the corresponding female category ($p<0.05$). A similar though not statistically significant, tendency existed when the durations of attack among the non-autopsied cases of each sex were compared.

The median survival time for the autopsied males was 8 minutes and for autopsied females 21 minutes. For the non autopsied cases the median survival time was 4 minutes among males and 38 minutes among females.

Table 6 shows the duration of the last attack by sex, age and autopsy status. In an analysis of this duration against age no significant trend was found for either sex among either the autopsied or non autopsied cases.

COMMENTS

For comparison of the duration of attack in respect of the category medically unattended deaths attributed to IHD no published figures were found which could be related to the findings in the present survey. Analyses have, however, been

TABLE 6. Medically unattended deaths by sex, age, duration of last attack and autopsy status

	Duration (minutes)				
	0—15		16—		
	No.	Per cent[1]	No.	Unknown No.	Total No.
Autopsied					
MALES					
Age 30—49	15	54	13	5	33
50—59	36	62	22	18	76
60—69	67	59	46	61	174
70—79	76	63	44	55	175
80—99	12	44	15	34	61
Total	206	60	140	173	519
FEMALES					
Age 40—59	7	50	7	7	21
60—69	17	45	21	32	70
70—79	27	47	30	55	112
80—99	14	50	14	25	53
Total	65	47	72	119	256
Non-autopsied					
MALES					
Age 40—59	7	70	3	4	14
60—69	20	74	7	14	41
70—79	13	59	9	15	37
80—99	7	58	5	1	24
Total	47	66	24	45	116
FEMALES					
Age 40—69	3	33	6	5	14
70—79	12	80	3	12	27
80—99	5	29	12	18	35
Total	20	49	21	35	76

[1] Of cases with known duration of last attack.

made of the time relation between the onset of acute symptoms and death from IHD in the population studies both from Belfast (McNeilly & Pemberton, 1968) and from Edinburgh (Fulton *et al* 1969) which covered deaths occurring both outside and in hospital. The agreement in respect of this time relation in the two reported studies is remarkably close and, on the basis of them, Fulton *et al.* (1969) considered that the mortality from IHD may be regarded as essentially an exponential function of time. This finding was based on an observation period of 4 weeks after the onset of attack.

As a further division of the duration of the last attack in the present survey was not considered possible when it exceeded two hours, an accumulation of such cases occurred in the last interval observed, which meant that the continuity of the distribution of the deaths was broken at this time. But, as shown in table 5 the time relation between the acute onset and death during the first two hours appeared as essentially the same exponential function as in the Belfast and Edinburgh studies.

The difference observed between the sexes in respect of duration of attack was reported also by McNeilly & Pemberton (1968) These authors found that the median survival time for males was 3 hours 30 minutes and for females 6 hours 18 minutes. The same authors found that a higher proportion of onsets in women were insidious In the Edinburgh study (Fulton *et al* 1969) it was found that, of all IHD deaths occurring within 4 weeks after the onset of the acute attack, 37 per cent of men and 27 per cent of women died within the first hour

McNeilly & Pemberton (1968) found that elderly men had a significant tendency to survive longer than younger men. A similar but not statistically significant, tendency was observed for the women in the same study It should be repeated that this observation was based on deaths both outside and in hospital As appeared from the study of the overall IHD mortality (Part I) the mean age both for men and women dying in hospital was higher than for the medically unattended deaths of each sex. This might be compatible with the fact that in older persons the duration of attack was in reality longer and, therefore, to a greater extent permitted admission to hospital. Another contributory cause of the higher mean age for hospital deaths might be that probably a larger number of the elderly individuals were already in hospital at the onset of symptoms of the fatal event, having been admitted with *some other condition.*

As appears from Part I (figs 1 and 2) the distribution of deaths among the three categories (medically unattended deaths, hospital deaths, and deaths in other institutions) was virtually reversed on comparison between the sexes among men medically unattended deaths were the largest category among women the smallest. An explanation of this observation in women is offered by the finding of a longer duration of the critical event, which to a greater extent permitted the institution of hospital care. The dominating category among women, deaths in other institutions (fig. 2) may be explained by the fact that women, owing to their greater life span, often live alone during their last years and for social reasons spend these years in such institutions, in which the onset of the terminal episode occurs.

SUMMARY

Of the 967 medically unattended deaths information concerning the duration of symptoms referable to the fatal attack was obtained for 595 (62 per cent) Among autopsied cases 60 per cent of the males and 47 per cent of the females died within 15 minutes of onset symptoms The corresponding figures for non-autopsied cases were for the males 66 per cent and for the females 49 per cent. The median survival time among autopsied cases was for men 8 minutes and for women 21 minutes among non-autopsied cases the median survival time for men was 4 minutes and for women 38 minutes.

No significant relation between age and duration of attack was found for either sex, either in autopsied or non autopsied cases.

B. ATTEMPT TO CALL FOR HELP DURING LAST ATTACK

During the studied period there was an emergency switchboard for telephone calls relating to acute cases staffed by experienced nurses serving Stockholm and the neighbouring communes of Lidingö Nacka, Solna and Sundbyberg. According to official population statistics (Stockholm Office of Statistics, 1969) the population in this area represented more than 80 per cent of the studied population.

Independently of this study and more than one year before it started, the nurses serving the emergency switchboard had been instructed to deal as quickly as possible with cases having suspected symptoms of acute heart disease. In cases when a physician could not be sent to the patient without delay the emergency switchboard operator arranged for an ambulance to bring the patient to hospital

RESULTS

Attempts to call for assistance during the last attack were considered to have been made in cases when measures were taken to summon help after the acute onset and while the patient was still conscious Of the total of 967 cases, reports of attempts to call for help in accordance with this criterion existed in 84 cases (9 per cent) Of these cases 66 were autopsied and 18 not. Only in one case was there a report that the patient himself had rung the emergency switchboard during the acute attack. There were also reports in two cases that, during the acute attack, the patient had called for help from relatives, who had then rung the emergency switchboard. In the other 81 (96 per cent) of the 84 cases in which an attempt to call for help was made during the acute attack, these measures had been taken by relatives or a witness No report existed of an attempt to call for help during the last attack among those found dead. Nor was there any report of an attempt during the acute attack to call the patient's own physician. With the exception of two cases the duration of attack exceeded 15 minutes in cases when an attempt to call for help was made during this attack.

Of the 84 persons who called for help during the acute attack 41 (49 per cent) died while waiting for a physician or ambulance. The death was considered to have occurred during transit to hospital when the patient was conscious at the time of departure but was found to be dead on arrival. Thirty-one cases (37 per cent) died according to this definition. In the remaining 12 cases (14 per cent) death occurred after they had been examined by a physician who had not considered immediate hospitalization necessary In three of these cases, according to witnesses, a diagnosis of respiratory infection had been made by the physician. No information as to the physician's diagnosis was available for the remaining cases of death after medical examination.

An account of the earlier state of health among cases in which help was called for during the last attack is given in the section on previous disorders.

COMMENTS

Both from Belfast (McNeilly & Pemberton, 1968) and from Edinburgh (Oliver 1968) it was found that, in the chain of events preceding admission to hospital, the longest delay is the time taken by the patient to call for help from a physician The two studies showed that the subsequent events represented a comparatively small part of the time before hospital care had been instituted.

In an investigation in Gothenburg (Lindström, 1969) a study was made of a group of IHD deaths occurring outside hospital and of a control group of patients who survived a clinically proven acute myocardial infarction. The two groups were studied in respect of measures taken to summon help in conjunction with the acute attack. Of the 18 cases occurring outside hospital, measures to summon help as soon as the acute symptoms appeared had been taken only in one. It was also found, both among those who died outside hospital and among the patients in the control group, that attempts to call for help were usually postponed for several hours after the onset of symptoms. The same authoress attempted also to analyse the reasons for the delay before help was summoned. She found

that some of the patients who died outside hospital tended to make light of or underestimate the significance of their symptoms, although in several cases they might have been aware of the importance of quickly calling for help She also suggested that in some cases there was a fear of being put in hospital. Another explanation of the omission to call for help by persons who died outside hospital was assumed to be that patients with earlier angina pectoris were so accustomed to their ailments that they could not distinguish between them and those which characterized the critical attack.

SUMMARY

Reports of attempts to call for help during the acute attack were obtained in only 84 cases (9 per cent) Of these, medical assistance was primarily sought by witnesses in 81 In a further 2 cases the patients called their relatives, who then took measures to summon medical aid. In only one case had this been done by the patient himself

In cases when help was called for during the acute attack 49 per cent of the deaths occurred before arrival of a physician or ambulance, 37 per cent during transit to hospital, and 14 per cent after examination by a physician. In one-quarter of the latter category of cases there were witnesses reports that an examining physician had diagnosed a respiratory infection. Immediate hospitalization had not been considered necessary for the remaining cases in this group.

C. PLACE OF OCCURRENCE OF DEATH AND ACTIVITY AT ONSET OF FATAL ATTACK

According to the definition of medically unattended death (Part I) these deaths occurred outside hospital or other institution for the chronically ill or aged. As, according to this definition, the place of occurrence of death was not exactly specified, an account of this is given below and also of the activity at onset of symptoms of the fatal attack.

DEFINITIONS

At home Death at home was considered to have occurred when the person died in his permanent dwelling or when temporarily resident elsewhere, e.g. in a hotel-room or in the home of acquaintances

At work Death occurred at the deceased's place of work.

During transit to hospital To this category were assigned deaths when, on departure for hospital, the person was conscious but was found to be dead on arrival in hospital.

Outside home All locations not classifiable in any of the abovementioned categories.

The activity at onset of symptoms of the fatal attack was considered unknown for unwitnessed cases. The recorded activities are shown in table 8

Asleep Waking with chest complaints referable to the fatal attack when bedrest and sleep had not been preceded by such symptoms.

At rest Activities such as lying down (but not sleeping) sitting and standing still.

Managing personal hygiene Activities such as eating, taking a shower or a bath, and visiting the toilet.

The group *Household and light occupational work* included a large variety of activities other than those classified among the other known activities in table 8

RESULTS

The distribution of the deaths by sex, place of occurrence, and autopsy status is shown in table 7 As seen from this table the majority of the deaths occurred at home. A rather larger proportion of women than men died at home among the autopsied cases the figure for men was 71 per cent and for women 79 per cent ($p<0.05$) while the corresponding percentages for non-autopsied cases did not differ significantly

As appears from the same table, deaths outside

TABLE 7 Medically unattended deaths by sex, place of occurrence and autopsy status

		At home	Outside home (except work)	At work	During transit to hospital	Total
Autopsied						
Males	No	366	105	31	17	519
	Per cent	71	20	6	3	100
Females	No	202	42	3	9	256
	Per cent	79	16	1	4	100
Non autopsied						
Males	No	92	21	2	1	116
	Per cent	79	18	2	1	100
Females	No.	63	8	1	4	76
	Per cent	83	11	1	5	100
Total	No.	723	176	37	31	967
	Per cent	75	18	4	3	100

home represented the second largest category for both sexes, both among autopsied and non-autopsied cases. The sexes did not significantly differ in this respect, either among autopsied or non-autopsied cases.

Table 7 shows that, of all 519 autopsied males, 31 (6 per cent) died at work, and of the non-autopsied cases 2 (2 per cent). The relative proportions of the two male categories dying at work were, however, not significantly different. In Sweden the general pensioning age is 67 years. Including non-autopsied cases the series consisted of 76 men below this age, of whom 28 (10 per cent) died at work. Of these 276 men below 67 years of age, however, 105 (38 per cent) were retired on early pension for various reasons. If the 28 deaths at work before general pensioning age are related to the remaining 171 able-bodied men of corresponding age, the proportion of male deaths at work was 16 per cent. Assuming that 40 hours per week were spent at work, and allowing for four weeks holiday and another two weeks of public holidays during the year, it could be estimated that 21 per cent of the year was spent at work. On the basis of this calculation, of the aforesaid 171 men 36 would have been expected to have died at work. The observed number of 28, however, does not differ significantly from that expected.

As appears from table 7, altogether 31 cases (3 per cent) died during transit to hospital. The relative proportions of the two sexes did not differ significantly in this respect, either among autopsied or non-autopsied cases, nor was there any difference between the autopsied and non-autopsied cases of the same sex.

Table 8 shows the distribution of deaths by sex, activity at onset of symptoms of the fatal attack,

TABLE 8. Medically unattended deaths by sex, activity at onset of last attack and autopsy status

	Autopsied				Non-autopsied				Total			
	Males		Females		Males		Females		Males		Females	
	No.	Per cent[1]	No.	Per cent	No.	Per cent	No.	Per cent[1]	No.	Per cent[1]	No.	Per cent[1]
Asleep	13	4	8	6	2	3	3	8	15	4	11	6
At rest	181	52	75	55	37	53	23	59	218	52	98	56
Managing personal hygiene	6	2	4	3	2	3	2	5	8	2	6	3
Household and light occupational work	21	6	11	8	8	11	2	5	29	7	13	7
Walking on level ground	72	21	29	21	14	20	8	21	86	21	37	21
Walking on level ground carrying burden	3	1	2	1	—	—	—	—	3	1	2	1
Walking upstairs or uphill	10	3	6	4	2	3	—	—	12	3	6	3
Running	7	2	—	—	1	1	—	—	8	2	—	—
Other heavy physical activity	7	2	—	—	2	3	—	—	9	2	—	—
Quarrel	10	3	1	1	—	—	—	—	10	2	1	1
Excitement or apprehension	7	2	1	1	2	3	1	3	9	2	2	1
Driving car	6	2	—	—	—	—	—	—	6	1	—	—
Alcohol intake	1		—	—	—	—	—	—	1		—	—
		100		100		100		100		100		100
Unknown	173		119		46		37		219		156	
Total	519		256		116		76		635		332	

[1] of cases with known activity at onset.

and autopsy status. As will be seen, this activity could be ascertained in altogether 592 (62 per cent) of the 967 cases.

In more than half of the cases the acute onset occurred *in conjunction with sleep or rest*. Of the 346 autopsied males, whose activity at onset was known, 194 (56 per cent) experienced the initial symptoms of the fatal attack during sleep or at rest, the figure for autopsied females being 83 (61 per cent). Of the non-autopsied cases whose activity at the acute onset was known, 39 (56 per cent) of the 70 men and 6 (67 per cent) of the 39 women incurred symptoms referable to the last attack while asleep or at rest. Taking the autopsied and non-autopsied cases together the sexes did not differ significantly in respect of symptoms of last attack arising while asleep or at rest nor was there any significant difference between autopsied and non-autopsied cases of the same sex.

For the other activities listed in table 8, there was no significant difference between the proportions of autopsied males and females nor between non autopsied cases of the same sex.

As appears from table 8 the acute onset came in 6 cases in conjunction with *car-driving*. In two of these cases there were witnesses reports from passengers concerning the course of events. In the remaining four cases the driver had shown no manifest signs of illness at the start of the drive. In 3 of the 6 cases the driver died while the car was in motion in the other 3 cases the car had stopped but the driver died while the engine was still running. All 6 cases were autopsied, and thus there were police reports containing witnesses reports from passengers or other road-users, and the police officer's own observations of the event. No serious injury occurred to persons involved, nor damage to vehicles, in any case.

In one of the cases in which the activity in conjunction with the acute onset was known, there was a witness report of alcohol consumption at the time. It appears, however that the quantity was moderate, and an analysis of alcohol in the body fluids was not made at autopsy. In another 6 cases, in which the activity at onset of the acute onset was insufficiently known, circumstances indicative of the influence of alcohol had occurred in conjunction with death. In these cases an analysis of alcohol was made in the postmortem and showed alcohol concentrations in heart blood varying between 60 and 310 mg %

The duration of the acute attack was analysed against the activity at onset. The results are given in table 9. This table compares cases in which the onset occurred *during* sleep or rest and those in which it occurred during some other known activity specified in table 8. As appears from table 9 89 (46 per cent) of the autopsied males whose onset came during sleep or rest died within 15 minutes of the onset, the corresponding figure for other activities being 117 (77 per cent). This difference is significant ($p<0.001$). For autopsied females in these categories the figures were, respectively 24 (29 per cent) of 83 and 41 (76 per cent) of 54 ($p<0.001$)

Of the non-autopsied cases 20 (51 per cent) of 39 men whose onset came during sleep or rest died within 15 minutes, the corresponding figure for other activities being 26 (84 per cent). This difference is significant ($p<0.01$). For non-autopsied women the figures were, respectively 9 (35 per cent) of 26 and 11 (85 per cent) of 13 ($p<0.01$)

COMMENTS

In a recent community study on IHD (Lindholm, 1969) the place where the onset of the symptoms of myocardial infarction occurred was recorded for a group of male survivors up to the age of 65. Of these patients 63 per cent got their first symptoms at home and 27 per cent at work, whereas 7 per cent experienced these symptoms while outside home and not at work. Of the male deaths in the present study 72 per cent occurred at home; for those for whom sufficient information existed on this point the place of occurence of death was also identical with that of onset of symptoms associated with the fatal attack. When comparing the findings in the two studies it must be kept in mind that no upper age limit was set in the present series. Of the 156 males in the present study aged 65 and below and not previously incapacitated for work,

TABLE 9 Medically attended deaths by sex duration of last attack activity at onset and autopsy status

		Duration (minutes)				
		0—15		16—	Unknown	Total
		No.	Per cent[1]	No.	No.	No.
Autopsied						
		MALES				
Asleep or at rest		89	46	105		
Other activity		117	77	35		
	Total	206	60	140	173	519
		FEMALES				
Asleep or at rest		24	29	59		
Other activity		41	76	13		
	Total	65	47	72	119	256
Non-autopsied						
		MALES				
Asleep or at rest		20	51	19		
Other activity		26	84	5		
	Total	46	66	24	46	116
		FEMALES				
Asleep or at rest		9	35	17		
Other activity		11	85	2		
	Total	20	51	19	37	76

[1] Of cases with known duration of last attack.

26 (17 per cent) sustained their final attack at work. The relative proportion of all males dying outside home and not at work in the present study (20 per cent) is higher than that found by Lindholm (1969) Again, this might be an effect of the dif ferent age groups covered by the studies as compared to the quoted study the present series comprised a large number of cases above the general pensioning age, with more time to spend outside home during office hours

The number of cases in which the activity at the acute onset was not considered to be known may seem relatively large. This is explained by the fact that witnesses reports concerning the activity at onset were required for this activity to be considered known. In the autopsied cases a police re port was available, and in several of these cases in which the actual death was not witnessed, the police reconstruction showed that death occurred in other situations than sleep or rest, in which the deceased probably sustained the acute attack.

Kuller *et al* (1966) in their study of sudden IHD deaths in Baltimore, found no relation between a specific activity and death. Their study related to persons of both sexes aged 40—64 years. In another American study of sudden IHD death among cases referred to the *Medical Examiner* Adelson & Hoffman (1961) found that in 21 per cent the onset came during sleep or rest and that 55 per cent of the cases suffered the acute attack during light activity No definition was, however given of light activity These authors also pointed out that attention must be paid to the group of cases which was selected from the sociomedical aspect, as the series consisted to a large extent of alcoholics and persons leading a vagabond exist ence. In another American Medical Examiner study of cases of sudden IHD death covering both sexes

and all ages, Weinberg & Helpern (1959) found that death occurred in 20 per cent of the cases during sleep or rest and that 37 per cent died during light everyday activities such as standing and conversing, but not walking.

Bainton & Peterson (1963) in their survey of IHD deaths in Seattle among persons up to 50 years of age, reported that 23 per cent survived sufficiently long during the last attack to be examined by a physician. These authors found, during the 48 hours preceding death, such situations as physical fatigue, emotional conflicts, agitation or trauma in 16 per cent of all cases. Very much more often, however there was a witness statement such as "he had had an unusually good day

In a study of autopsied cases of death in IHD among military personnel aged 18 and upwards Yater *et al* (1951) found that the acute attack more often occurred during severe physical effort in young persons. Conversely these authors found that the proportion of cases occurring during rest increased with age. They also reported that in roughly one-third of cases the acute attack occurred during mild activity though this concept was not defined.

Pell & D Alonzo (1964) in a study of men in earning occupations, found that in 60 per cent of the IHD deaths the onset occurred during sleep or rest.

Stevens (1963) in a study of a large hospital series of myocardial infarctions, found that in 42 per cent of cases the onset came during sleep or rest, 48 per cent being engaged on light everyday work, 7 per cent heavy physical work, and 3 per cent in connection with mental stress. The cited study covered all ages, with activities fairly uniformly distributed over the various ages.

Electrocardiographic (ECG) recording in conjunction with car-driving, both of patients with earlier diagnosed IHD and persons with presumably normal hearts, showed an accentuation in relation to the recording at rest of signs of myocardial ischaemia or the appearance of such signs (Taggart & Gibbons, 1967 Bellet *et al* 1968 Simonson *et al* 1968 Somerville *et al* 1968 Taggart *et al* 1969) In persons with apparently normal hearts Taggart & Gibbons (1967) recorded rapid heart rates and in some cases abnormal ST and T changes in the ECG during driving in busy London traffic. From a study including healthy subjects as well as patients with previously diagnosed IHD Simonson *et al* (1968) reported that significant ST depression and T wave changes in the ECG were recorded during driving in both categories of cases, but more frequently in the latter Bellet *et al* (1968) found that, among subjects with previously diagnosed IHD 17 per cent reacted to driving with ECG changes suggesting myocardial ischaemia. ECG changes indicative of myocardial ischaemia were recorded in an even higher proportion of subjets studied by Somerville *et al* (1968) and by Taggart *et al* (1969) In the latter study ECG recordings showed ST changes not secondary to a more rapid heart rate during driving among 10 per cent of the, as far as was known, healthy drivers. Of the subjects in the same study with earlier diagnosed IHD the ST and T changes recorded during rest were accentuated during driving in half of the cases, and in another one-quarter of cases multiple ventricular ectopic beats appeared some of which multifocal, and in one case even a short run of ventricular tachycardia. Among the drivers with earlier diagnosed IHD angina pectoris and signs of left heart failure were reported during driving in some cases

Happily traffic accidents due to loss of consciousness caused by IHD have been reported to be rare. Peterson & Petty (1962) and Myerburg & Davis (1964) in their American Medical Examiner series, found loss of consciousness due to IHD an unusual cause of fatal accidents. In a Swedish survey by Ysander (1969) out of 1000 traffic accidents only one was caused by sudden illness of the driver

Conway (1968) after consumption of moderate quantities of alcohol by patients with earlier diagnosed IHD found both a deterioration of several haemodynamic parameters and ECG signs indicative of myocardial ischaemia. Regan *et al* (1965) in experiments with alcoholists with, as far as was known, normal hearts, found that the administration of alcohol was followed by haemodynamic

signs of reversible impairment of the left heart function. By reason of two male cases of sudden death in which autopsy revealed advanced but not occlusive arteriosclerotic lesions in the coronary arteries, simultaneously with an alcohol concentration in the blood exceeding 200 mg % Heggtveit (1965) discussed whether alcohol might have been a contributory factor in these deaths It was suggested by this author that the combined effects of alcohol, including (a) dilation of peripheral arterioles and (b) constriction of the coronary arteries reported to occur at blood levels above 100 mg % (Burch & Walsh, 1960) in addition to (c) the increase in oxygen consumption of the heart under the influence of alcohol reported by Genz (1963) may have significantly affected the fate of his cases Autopsy was done in all six cases in the present study with blood levels of alcohol between 60 and 310 mg % and according to the pathologist the following findings were made: old myocardial infarct (4 cases) diffuse myocardial fibrosis (1) and in the remaining case coronary arteriosclerosis without report of myocardial lesions.

The consistent tendency of a shorter duration of attack during other activities than sleep or rest may possibly be a consequence of the fact that the attack is then experienced more dramatically and distinctly by a witness, and that therefore an attack of a more insidious character may fall into the background. In the same way in the case of an attack arising during sleep or rest, one may imagine that a witness would to a greater extent describe a gradual onset In cases when the acute onset was considered to have taken place during relatively heavy activities, however such as walking or running, it appears improbable that these activities would have been initiated after the onset of chest pain and/or dyspnoea.

SUMMARY

Of deaths outside hospital most occurred at home of autopsied males 71 per cent died at home against 79 per cent of autopsied females, the figures for non-autopsied cases being 79 and 83 per cent respectively Of all men below 67 years (the general pensionable age) in the survey 9 per cent died at work.

In the majority of cases in which the activity at onset of the fatal attack was known, the attack occurred during sleep or rest. This applied to both sexes and to autopsied as well as non-autopsied cases

In 6 cases the acute onset came during car-driving, but without serious injury to persons or damage to vehicles.

The activity at onset has been compared with the duration of attack. For both sexes, and for autopsied as well as non autopsied cases, there was a significant tendency to longer duration of attack during sleep or rest than during other activities. A conceivable contributory reason for this finding might be that an attack during other activities than sleep or rest is more readily considered by a witness to be more acute, with the result that preceding symptoms attributable to the fatal event fell into the background.

D PREVALENCE OF A HISTORY OF CERTAIN PREVIOUS DISORDERS

METHODS AND DEFINITIONS

Of the medically unattended deaths attributed to ischaemic heart disease (IHD) included in the present survey 80 per cent underwent postmortem examination at the Government Institute for Forensic Medicine, Karolinska Institutet. As already noted (p 23) in these cases a copy of the police report was always available as basis for the medical examiner's judgment of the extent of examination required. A part of the training received by police officers responsible for these investigations is a course at the Institute for Forensic Medicine on the common causes of death which the police have to investigate.

The police reports nearly always contained witnesses statements concerning the earlier state of health of the deceased. When a witness had expressly stated in a police report that the deceased had earlier been healthy and had not been under medical treatment, this statement was accepted without further investigation. On the other hand if the police report contained no statement concerning the deceased's earlier state of health, or if the description was too meagre, an enquiry was made to relatives or to the Social Insurance Office. When the physician in charge was named in the report, he was asked about the deceased's earlier state of health. This procedure was followed also in all non-autopsied cases, i.e. in which the death certificate was issued by the physician in charge. In cases reported to have been under earlier hospital care the hospital records were called for.

The following diagnoses were recorded

Myocardial infarction The diagnosis was accepted when established during treatment in hospital or by a physician on the basis of available clinical information. This category also included cases in which this diagnosis, on the basis of corresponding information, was judged to be "probable or suspect by the physician concerned.

Angina pectoris Diagnosis defined as treatment with a nitroglycerine compound.

Heart failure Diagnosis defined as treatment with digitalis.

Hypertension Diagnosis defined as medicamental treatment aimed at lowering the blood pressure.

Diabetes Clinically manifest diabetes mellitus.

Other disorders In the absence of any of the aforesaid diagnoses the occurrence of the following diseases reported by a physician was recorded hyperlipaemia, gout, and chronic respiratory disease. These diagnoses were mutually exclusive.

Suspect IHD manifestations In the absence of the aforesaid diagnoses cases were assigned to this category when, according to relatives, the person had complained of pain in the chest or an unusual dyspnoea without a physician being consulted for these complaints.

None of the preceding disorders To this group were ascribed cases in which relatives had expressly stated that the deceased had earlier been healthy. If contact with a physician had taken place, the latter was questioned concerning the occurrence of the preceding disorders. If such occurrence was denied, the case was assigned to this group

Unknown No information obtained concerning earlier state of health.

By prevalence of a certain disorder is meant in the sequel the prevalence of a history of the disorder concerned

RESULTS

Previous disorders in relation to sex and age

Table 10 shows the prevalence of the specified disorders in relation to age for the autopsied and non-autopsied cases by sex.

Among the men, as appears from the table, a previous clinical diagnosis of myocardial infarction occurred in 18 per cent of the autopsied and 28 per cent of the non-autopsied cases ($p<0.05$). Angina pectoris had been diagnosed in a significantly ($p<0.001$) larger proportion of non-autopsied than of autopsied cases (61 per cent against 26 per cent). Heart failure was the commonest diagnosis among the autopsied cases it was present in 32 per cent, and in non-autopsied cases 66 per cent ($p<0.001$). A diagnosis of hypertension was obtained for 11 per cent of the

TABLE 10 *Medically unattended deaths by sex, age, autopsy status and prevalence of certain previous disorders*

	Total N	Myocardial infarction No.	Myocardial infarction Per cent	Angina pectoris N	Angina pectoris Per cent	Heart failure No.	Heart failure Per cent	Hypertension No.	Hypertension Per cent	Diabetes No	Diabetes Per cent	Suspect IHD symptoms No.	Suspect IHD symptoms Per cent	Other disorders No.	Other disorders Per cent	None of previously mentioned disorders No	None of previously mentioned disorders Per cent
Autopsied																	
MALES																	
Age 30—49	33	8	24	9	7	6	18	2	6	3	9	8	24	—	—	8	24
50—59	76	18	24	19	25	17	22	9	12	3	4	12	16	1	1	26	34
60—69	174	33	19	44	25	51	29	26	15	21	12	21	12	5	3	40	23
70—79	173[1]	29	17	52	30	73	42	22	13	18	10	16	9	4	2	46	27
80—99	61	6	10	8	13	17	28	—	—	3	5	8	13	1	2	27	44
Total	517[1]	94	18	132	26	164	32	59	11	48	9	65	13	11	2	147	28
FEMALES																	
Age 40—59	21	2	10	6	29	—	—	6	29	—	—	3	14	—	—	8	38
60—69	70	13	19	16	23	26	37	16	23	9	13	11	16	1	1	17	24
70—79	112	11	10	33	29	44	39	28	25	20	18	18	16	1	1	25	22
80—99	52[2]	7	13	13	25	29	56	9	17	1	2	6	12	—	—	12	23
Total	255[2]	33	13	68	27	99	39	59	23	30	12	38	15	2	1	62	24
Non-autopsied																	
MALES																	
Age 40—59	14	7	50	10	71	8	57	5	36	—	—	—	—	—	—	—	—
60—69	41	16	39	28	68	24	59	10	24	5	12	3	7	1	2	1	2
70—79	37	7	19	20	54	26	70	8	22	5	14	1	3	1	3	1	3
80—99	24	3	13	13	54	19	79	6	25	2	8	2	8	—	—	—	—
Total	116	33	28	71	61	77	66	29	25	1	10	6	5	2	2	2	2
FEMALES																	
Age 40—69	14	4	29	7	50	11	79	7	50	—	—	—	—	—	—	—	—
70—79	27	3	11	13	48	16	59	13	48	8	30	1	4	—	—	1	4
80—99	35	—	—	11	31	20	57	15	43	4	11	3	9	—	—	3	9
Total	76	7	9	31	41	47	62	35	46	12	16	4	5	—	—	4	5

[1] Two cases with insufficient data excluded.
[2] One case with insufficient data excluded.

autopsied cases and 25 per cent of the non autopsied ($p<0.001$) The prevalence of diabetes in the two categories of male cases did not differ significantly· the same applied to "other disorders" Suspect IHD symptoms were more frequently recorded for autopsied than non autopsied cases (13 per cent and 5 per cent, respectively $p<0.05$) A negative history of any of the abovementioned disorders was obtained for 28 per cent of the autopsied cases and 2 per cent of the non autopsied cases ($p<0.001$)

Table 10 shows, in the same way as for men, the prevalence of the various disorders among women. As regards the prevalence of a history of previous clinically recognized myocardial infarction, no significant difference existed between autopsied and non autopsied cases. Angina pectoris had been diagnosed more frequently among non autopsied than among autopsied cases (41 per cent against 27 per cent, $p<0.05$) As among the men, heart failure was the commonest diagnosis among the women, and more common in the non-autopsied than in the autopsied cases (62 per cent against 39 per cent, $p<0.001$) Hypertension was likewise commoner among the non autopsied cases (46 per cent) than among the autopsied (23 per cent, $p<0.001$) The relative proportions of diabetics in the two categories did not differ significantly Suspect IHD symptoms were recorded for 15 per cent of the autopsied cases and 5 per cent of the non autopsied ($p<0.05$) A negative history with respect to the abovementioned disorders as well as other disorders (table 10) was obtained for 24 per cent of the autopsied cases and 5 per cent of the non-autopsied ($p<0.001$)

The prevalence of a history of the disorders listed in table 10 when compared by sex among the autopsied cases, yielded a significantly ($p<0.001$) higher prevalence rate of hypertension in the females (23 per cent) as compared to 11 per cent in the males. No such difference was found for the prevalence of any of the other disorders (including the category none of the preceding disorders) for the autopsied cases.

The corresponding comparison between the non-autops ed cases showed significant differences between the sexes for the prevalence of a history of any of the following disorders myocardial infarction, angina pectoris, and hypertension ($p<0.01$ for all three) Thus, a history of previous myocardial infarction was obtained for 28 per cent of the males as compared to 9 per cent of the females angina pectoris had been diagnosed in 61 per cent of the male cases and in 41 per cent of the female, whereas hypertension was found to be a more frequent diagnosis in the females (46 per cent as compared to 25 per cent in the males) There was no significant sex difference among non-autopsied cases with respect to prevalence of any of the remaining diagnoses shown in table 10

In relation to age there was a tendency to a greater prevalence ($p<0.05$) of earlier diagnosed myocardial infarction in lower age groups among the autopsied males (table 10) The reverse was observed for heart failure, which was more frequent in the higher age groups ($p<0.01$) For the remaining disorders in table 10 no significant age trend was observed among autopsied male cases.

As among the autopsied male cases, in non autopsied cases of the same sex (table 10) a previous diagnosis of myocardial infarction was commoner in the lower age groups ($p<0.01$) Among the non autopsied males there was no significant age trend for a history of any of the other disorders recorded in table 10

Among the women the prevalence of heart failure increased significantly ($p<0.001$) with rising age among the autopsied cases The prevalence of none of the other disorders listed in table 10 showed any significant age trend among this category of women. Among the non-autopsied females there was a significant ($p<0.01$) age trend only for previous clinically recognized myocardial infarction, which was more common in the lower age groups.

The prevalence of a history of one and pairs of two concomitant diagnoses not recorded as mutually exclusive is shown by sex and autopsy status in table 11 Table 12 shows the concomitant prevalence of two disorders not recorded as mutually exclusive by sex, age, and autopsy status.

As appears from these tables, the pairwise com-

TABLE 11 Medically unattended deaths by sex autopsy stat and pr alenc f one and two previous disorders

	AUTOPSIED				NON AUTOPSIED			
	Males		Females		Males		Females	
	No.	Per cent of total	No.	Per cent of total	No	Per cent of total	No.	Per cent of total
Total	517[1]		255[2]		116		76	
Myocardial infarction								
All	94	18	33	13	33	28	7	9
With angina pectoris	53	10	18	7	26	22	3	4
With heart failure	54	10	28	11	25	22	6	8
With hypertension	8	2	10	4	5	4	4	5
With diabetes	7	1	3	1	3	3	1	1
Angina pectoris								
All	132	26	68	27	71	61	31	41
With heart failure	59	11	48	19	47	41	16	21
With hypertension	27	5	27	11	16	14	17	22
With diabetes	12	2	8	3	7	6	5	7
Heart failure								
All	164	32	99	39	77	66	47	62
With hypertension	30	6	31	12	22	19	22	29
With diabetes	21	4	13	5	8	7	6	8
Hypertension								
All	59	11	59	23	29	25	35	46
With diabetes	6	1	9	4	4	3	7	9
Diabetes all	48	9	30	12	12	10	12	16

[1] Two cases with insufficient data excluded.

[2] One case with insufficient data excluded.

binations of diagnoses—like the individual diagnoses—were throughout commoner among the non-autopsied than among the autopsied cases of both sexes. Thus among the autopsied male cases a history of myocardial infarction together with angina pectoris was obtained for 10 per cent, as compared to 22 per cent for the corresponding non-autopsied cases ($p<0.001$) Likewise, among the non-autopsied compared with autopsied male cases there was a higher prevalence of the following other combinations recorded in table 11 myocardial infarction and heart failure (22 per cent vs. 10 per cent, $p<0.01$) angina pectoris and heart failure (41 per cent vs 11 per cent, $p<0.001$) angina pectoris and hypertension (14 per cent vs. 5 per cent, $p<0.01$) and heart failure and hypertension (19 per cent vs. 6 per cent, $p<0.001$) The prevalence of the combination heart failure and diabetes did not significantly differ between the two categories of male deaths.

A comparison between the two categories of female deaths of the concomitant prevalence of the pairs of disorders listed in tables 11 and 12 showed that the following combinations were more frequently recorded for the non-autopsied than for the autopsied cases angina pectoris and hypertension (22 per cent vs 11 per cent, $p<0.05$) and heart failure and hypertension (29 per cent vs 12 per cent, $p<0.001$) None of the other pairs of disorders was found to differ significantly with respect to prevalence in the females when compared by autopsy status

A comparison of the prevalence of the pairs of diagnoses in tables 11 and 12 between autopsied cases by sex showed that the following pairs of disorders were significantly more frequently re-

TABLE 1 Medically unattended deaths by sex, age, autopsy status and concomitant prevalence of two previous disorders

	Total No	Myocardial infarction & angina pectoris No	Myocardial infarction & angina pectoris Per cent	Myocardial infarction & heart failure No	Myocardial infarction & heart failure Per cent	Angina pectoris & heart failure No	Angina pectoris & heart failure Per cent	Angina pectoris & hypertension No	Angina pectoris & hypertension Per cent	Heart failure & hypertension No.	Heart failure & hypertension Per cent	Heart failure & diabetes No.	Heart failure & diabetes Per cent
Autopsied													
MALES													
Age 30—49	33	5	15	4	12	1	3	—	—	—	—	1	3
50—59	76	12	16	9	12	8	11	4	5	1	1	—	—
60—69	173	15	9	17	10	16	9	8	5	15	9	8	5
70—79	175	17	10	20	12	31	18	15	9	14	8	11	6
80—99	61	4	7	4	7	5	3	—	—	—	—	1	2
Total	517[1]	53	10	54	10	59	11	27	5	30	6	21	4
FEMALES													
Age 40—59	21	2	9	—	—	—	—	2	9	—	—	—	—
60—69	70	5	7	10	14	11	16	6	9	8	11	4	6
70—79	112	7	6	11	10	27	24	14	13	17	15	9	8
80—99	52[2]	4	8	7	13	10	19	5	10	6	12	—	—
Total	255[2]	18	7	28	11	48	19	27	11	31	12	13	5
Non-autopsied													
MALES													
Age 40—59	14	4	29	6	43	5	36	3	21	3	21	—	—
60—69	41	14	34	11	27	18	44	6	15	8	20	2	5
70—79	37	6	16	6	16	14	38	4	11	5	14	4	11
80—99	24	2	8	2	8	10	42	3	13	6	25	2	8
Total	116	26	22	25	22	47	41	16	14	22	19	8	7
FEMALES													
Age 40—69	14	2	14	3	21	4	29	6	43	5	36	—	—
70—79	27	1	4	3	11	6	22	5	19	9	33	4	15
80—99	35	—	—	—	—	6	17	6	17	8	23	2	6
Total	76	3	4	6	8	16	21	17	22	22	29	6	8

[1] Two cases with insufficient data excluded. [2] One case with insufficient data excluded.

TABLE 13 Medically attended deaths by sex duration of last attack and previous disorders Autopsied cases

	Duration (minutes)					
	0—15		16—			
	No.	Per cent[1]	No.	p[2]	Unknown No.	Total No.
MALES						
Myocardial infarction	44	60	30	N.S.	20	94
Angina pectoris	69	71	28	<0 01	35	132
Heart failure	70	67	35	N S.	59	164
Hypertension	6	68	1	N S.	21	59
Diabetes	19	58	14	N S	15	48
Suspect IHD symptoms	25	56	20	N.S.	20	65
Other disorders	5		4	N S	2	11
None of abovementioned disorders	45	51	44	<0 05	58	147
Total subjects	205	59	140		172	517[3]
FEMALES						
Myocardial infarction	11	58	8	N S	14	33
Angina pectoris	19	51	18	N S.	31	68
Heart failure	34	63	20	<0 01	45	99
Hypertension	16	50	16	N S.	27	59
Diabetes	10	48	11	N.S.	9	30
Suspect IHD symptoms	5	25	15	N.S.	18	38
Other disorders	1		—	N S.	1	2
None of abovementioned disorders	13	46	15	N.S.	34	62
Total subjects	65	47	72		118	255[4]

[1] Of cases with known duration of last attack

[2] Denotes significance of difference between proportions at duration 0—15 and 16— minutes in presence and, respectively absence of the disorder N S.=not significant ($p > 0.05$)

[3] Tw… …ases with insufficient data excluded.

[4] One case w…th insufficient data excluded.

corded among females than among males angina pectoris and heart failure (19 per cent vs. 11 per cent) angina pectoris and hypertension (11 per cent vs 5 per cent) and heart failure and hypertension (12 per cent vs 6 per cent, $p < 0.01$ for all three combinations) No significant sex difference was recorded as regards the prevalence of the remaining pairs of disorders shown in tables 11 and 12 for the autopsied cases

The same comparison between the non-autopsied cases of the same sex (table 11) showed that the following combinations of previous disorders were recorded more frequently for males than for females myocardial infarction and angina pectoris (22 per cent vs. 4 per cent, $p < 0.01$) myocardial infarction and heart failure (22 per cent vs 8 per cent, $p < 0.05$) and angina pectoris and heart failure (41 per cent vs 21 per cent, $p < 0.01$) No significant sex difference was recorded as regards the prevalence of the remaining pairs of disorders shown in tables 11 and 12 for the non-autopsied cases.

Among the autopsied cases no significant age trend was found for any of the pairs of diagnoses in table 12, either for men or women. Among the non-autopsied cases, both among men and women, the following pairs of diagnoses were represented to a greater extent among younger cases myocardial infarction and angina pectoris ($p < 0.05$ for both sexes) and myocardial infarction and heart failure

TABLE 14 Medically unattended death by sex duration of last attack and previous disorder Non-autopsied cases

	Duration (minutes)					
	0—15		16—			
	N	Per cent[1]	No	p[2]	Unknown No	Total No.
MALES						
Myocardial infarction	18	86	3	<0.05	12	33
Angina pectoris	33	72	13	N.S.	25	71
Heart failure	34	76	11	N.S.	32	77
Hypertension	10	56	8	N.S.	11	29
Diabetes	3		1	N.S.	8	12
Suspect IHD symptoms	—	—	2	N.S.	4	6
Other disorders	—	—	1	N.S.	1	2
None of abovementioned disorders	1		1	N.S.	—	2
Total subjects	47	66	24		45	116
FEMALES						
Myocardial infarction	1		2	N.S.	4	7
Angina pectoris	9	56	7	N.S.	15	31
Heart failure	14	56	11	N.S.	22	47
Hypertension	12	60	8	N.S.	15	35
Diabetes	5		1	N.S.	6	12
Suspect IHD symptoms	—	—	2	N.S.	2	4
Other disorders	—	—	—		—	—
None of abovementioned disorders	—	—	3	N.S.	1	4
Total subjects	20	49	21		35	76

[1] Of cases with known duration of last attack.

[2] Denotes significance of difference between proportions at duration 0—15 and 16— minutes in presence and, respectively absence of the disorder N.S. = not significant ($p>0.05$)

($p<0.01$ for both sexes) No significant age trend existed for any of the remaining pairs of diagnoses among non-autopsied cases.

Previous disorders in relation to duration of last attack

Table 13 shows the distribution of the autopsied cases by sex, duration of last attack, and previous disorders. The corresponding distribution of the non-autopsied cases is shown in table 14. Among the autopsied males, as seen from table 13 the proportion of deaths within the first 15 minutes after onset of the fatal event was significantly ($p<0.01$) higher in the presence of a previous diagnosis of angina pectoris than in the absence of this diagnosis. On the other hand a negative history of any of the disorders shown in this table was found to promote a longer survival. For the remaining diagnoses among males in table 13 no significant relation with the duration of the fatal attack was found.

Among the autopsied females (table 13) a significantly ($p<0.01$) larger proportion died within 15 minutes in the presence of a history of heart failure than in the absence of this diagnosis. The presence of any of the other diagnoses shown in table 13 was not found to be associated with any significantly different duration of the last attack.

Of the non-autopsied males (table 14) for whom the duration of the last attack could be assessed,

TABLE 15 Medically unattended deaths by sex, duration of last attack and two concomitant disorder Autopsied cases

	Duration (minutes)					
	0—15		16—			
	No.	Per cent[1]	No.	p[2]	Unknown No.	Total No.
MALES						
Myocardial infarction & angina pectoris	30	71	12	N.S.	11	53
Myocardial infarction & heart failure	27	64	15	N.S	12	54
Angina pectoris & heart failure	29	76	9	<0 05	21	59
Angina pectoris & hypertension	16	89	2	<0 05	9	27
Heart failure & hypertension	13	68	6	N.S.	11	30
Heart failure & diabetes	6	43	8	N.S.	7	21
Total subjects with known previous disorders	205	59	140		172	517
FEMALES						
Myocardial infarction & angina pectoris	5	50	5	N.S.	8	18
Myocardial infarction & heart failure	9	60	6	N.S.	13	28
Angina pectoris & heart failure	14	58	10	N.S	24	48
Angina pectoris & hypertension	8	57	6	N.S.	13	27
Heart failure & hypertension	12	63	7	N.S.	12	31
Heart failure & diabetes	5		3	N.S.	5	13
Total subjects with know previous disorders	65	47	72		118	255

[1] Of cases with known duration of last attack.
Denotes significance of difference between proportions at duration 0—15 and 16— minutes in presence and, respectively absence of the pair of disorders. N.S.=not significant ($p>0.05$)

18 (86 per cent) of the 21 cases with a previous diagnosis of myocardial infarction succumbed within 15 minutes of onset of symptoms of the critical attack as compared to 29 (58 per cent) of the 50 cases without a history of myocardial infarction ($p<0.05$)

For the remaining disorders shown in table 14 no significant correlation could be demonstrated for the non autopsied cases of either sex between the presence or absence of the disorders concerned and the duration of the fatal attack.

In the same way as for the previously diagnosed single disorders, the distribution by sex, duration of the fatal event, and two concomitant disorders are shown for the autopsied cases in table 15 and for the non-autopsied cases in table 16. As appears from these tables, there was an almost significant

TABLE 16. Medically unattended deaths by sex, duration of last attack and concomitant disorders. Non-autopsied cases

	Duration (minutes)					
	0—15		16—			
	No.	Per cent	No.	p[a]	Unknown No.	Total No.
MALES						
Myocardial infarction & angina pectoris	13	88	2	N.S.	9	26
Myocardial infarction & heart failure	13	81	3	N.S.	9	25
Angina pectoris & heart failure	22	79	6	N.S.	19	47
Angina pectoris & hypertension	6	60	4	N.S.	6	16
Heart failure & hypertension	8	53	7	N.S.	7	22
Heart failure & diabetes	1		1	N.S.	6	8
Total subjects with known previous disorders	47	66	24		45	116
FEMALES						
Myocardial infarction & angina pectoris	1		1	N.S.	1	3
Myocardial infarction & heart failure	1		1	N.S.	4	6
Angina pectoris & heart failure	5		2	N.S.	9	16
Angina pectoris & hypertension	5		4	N.S.	8	17
Heart failure & hypertension	9	64	5	N.S.	8	22
Heart failure & diabetes	3		—	N.S.	3	6
Total subjects with known previous disorders	20	49	21		35	76

Of cases with known duration of last attack

[a] Denotes significance of difference between proportions at duration 0—15 and 16— minutes in presence and, respectively, absence of the pair of disorders. N.S. = not significant ($p > 0.05$)

($p < 0.05$) tendency to shorter survival only in the presence of the following pairs: angina pectoris and heart failure, angina pectoris and hypertension. It is also seen that this finding was confined to the category of autopsied males. The presence or absence of any of the other pairs of disorders did not significantly affect the duration of the fatal event either for the autopsied or non-autopsied cases of either sex.

Call for medical assistance during last attack in relation to previous diagnoses

As earlier mentioned (p. 29) there were reports of assistance being called for during the last attack, indicating that the person was still alive, in altogether 84 cases.

The frequency of all calls for medical assistance in relation to previous diagnoses is shown in table 17.

TABLE 17 Medically unattended deaths Call for medical assistance during last attack among patients with recorded previous disorders (n=84)

	Total No.	Call for medical assistance No.	Per cent
Myocardial infarction	167	11	7
Angina pectoris	302	22	7
Heart failure	387	27	7
Hypertension	182	13	7
Diabetes	102	8	8
Suspect IHD symptoms	113	12	11
Other disorders	15	1	7
None of abovementioned disorders	215	21	10

It is seen from this table that there was no striking over or underrepresentation of any of these diagnoses with respect to attempts to seek medical assistance during the fatal attack, and the relative proportion of such attempts was not found to differ significantly in the presence or absence of any specific diagnosis.

COMMENTS

The criteria used for the various diagnoses in the present survey had to be set with regard to the fact that with few exceptions the studied cases had not been seen alive by the author For example the now usual criteria of acute myocardial infarction (typical history accompanied by characteristic electrocardiographic and biochemical abnormalities) could not be used in several cases in which the diagnosis was made without access to the necessary laboratory resources *It is also probable that, in* the event of a uniform clinical evaluation of the condition of the patients prior to death, some of the diagnoses of angina pectoris, heart failure and hypertension made in accordance with the used criteria would have been regarded as false positive It appears probable, however that such an evaluation would have resulted in at least as many false negatives for these diagnoses. Thus, of the autopsied cases in the present survey suspect symptoms —but not confirmed by a physician—suggestive of IHD were recorded for 13 per cent of the males and for 15 per cent of the females. No published results exist for comparison of the occurrence of previously diagnosed disorders in cases of death attributed to IHD occurring outside hospital, in which clear definitions had been given of these previous disorders.

The consistently higher prevalence of the recorded disorders in the non-autopsied cases would appear to be due to the fact that non autopsied cases were to a greater extent under treatment for one of these disorders by the physician who issued the death certificate and thus this category could be expected to be more ill than the autopsied cases. This will be dealt with later on in conjunction with the discussion of the validity of the IHD diagnosis (section F)

Myerburg & Davis (1964) found, in a U.S. material of sudden IHD deaths (defined as occurring within 24 hours of onset of symptoms) among white subjects aged 65 years and younger a previous history of IHD for 26 per cent of the males and for 22 per cent of the females The same authors reported that previous but not diagnosed symptoms suggestive of IHD were present in a further 34 per cent of the males and 37 per cent of the females

In an epidemiological survey from Baltimore of IHD deaths in the ages 40—64 years Kuller *et al* (1966) found that, of the white males who died within 24 hours of onset of symptoms 53 per cent had had a history of heart disease, 24 per cent had previously diagnosed hypertension, 12 per cent had a history of diabetes, whereas 32 per cent had been free of any of these diseases or other cardiovascular disease. *The same authors were able to record* among white females dying within 24 hours of onset of symptoms a history of heart disease for 44 per cent, of hypertension for 42 per cent, and of diabetes for 8 per cent.

The quoted study of Myerburg & Davis (1964) related solely to cases referred to the Medical Examiner and it may therefore be assumed that in this series the lower socio-economic groups were over represented. This basis for selection may account for the relatively high proportion of cases with previous but not diagnosed symptoms suggestive of

IHD The prevalence rates given by Kuller *et al* (1966) for hypertension are of interest, since they confirm the sex difference found in the present study The prevalence of a history of diabetes reported by them also roughly compares with the present finding. The prevalence of heart disease reported by these authors is difficult to relate to the findings in the present study as no exact definition of the implication of this diagnosis was given.

Fulton *et al* (1969) in their community study of IHD in Edinburgh, found that, of the deaths occurring within 24 hours of onset of acute symptoms, either a definite history of or symptoms suggestive of IHD were recorded for 53 per cent of the cases and that 30 per cent had had at least one previous clinically diagnosed myocardial infarction. This study was limited to subjects up to the age of 70 years. Of all cases included in the present study under the age of 70 a history of myocardial infarction could be obtained for 23 per cent.

Bainton & Peterson (1963) reporting from their study on IHD deaths in persons 50 years of age and younger in Seattle, U.S.A., found that, of those dying within 24 hours of onset of symptoms of acute illness, 41 per cent had had no symptoms or signs referable to the cardiovascular system. In comparison with the present series of autopsied males this figure appears relatively high of the autopsied males in the present series under the age of 50 the death had in 8 of 33 cases (24 per cent) not been preceded by any of the following disorders myocardial infarction, angina pectoris, heart failure, hypertension, diabetes, suspect IHD symptoms or other disorders as defined previously (p 37)

An interesting report is that of McNamee *et al* (1970) from Belfast. These authors found that, of 160 patients who survived ventricular fibrillation complicating acute IHD documented evidence of previous myocardial infarction existed for 29 per cent, and of angina pectoris without previous infarction for 36 per cent, whereas there was no previous evidence of IHD for 36 per cent. In the quoted study no upper age limit was used

As far as is known, it has not earlier been reported in conjunction with medically unattended IHD deaths that a history of previous myocardial infarction is more common among younger age groups An explanation of this finding may be that during the acute infarction episode the hospital mortality is lower for younger individuals, as shown by among others, Biörck *et al* (1957) A relatively larger proportion of younger persons will then be exposed to the risk of fatal recurrence after discharge from hospital.

McNeilly & Pemberton (1968) in their community study from Belfast of fatal cases attributed to IHD found that older men in their second or subsequent attack tended to survive longer This observation could not be confirmed in the present study of medically unattended deaths. However as was shown previously (p 18) the mean age of the male hospital deaths was higher no record of previously diagnosed disorders was made for this category of cases

Sievers (1963) in his study of hospitalized cases of acute myocardial infarction noticed a higher four week mortality for subjects of each sex with a previously diagnosed infarction than for cases without this diagnosis in spite of comparable distributions by age for both categories of cases.

In a group of patients with angina pectoris observed by Semm (1960) for periods varying between 5 and 25 years, sudden death attributed to IHD occurred in 37 per cent. However no definition was given of what was meant by sudden death in this study Sievers (1963) concluded from his study of cases of acute myocardial infarction surviving long enough to reach hospital that antecedent angina pectoris did not make any difference to the short-term, defined as four week, outlook after infarction. This observation is of interest in the light of the findings in the present survey in which the cases are not included in any hospital statistics. Of the autopsied males, who constituted the largest category in this survey there was a significantly larger proportion of deaths within 15 minutes in the presence than in the absence of a diagnosis of angina pectoris This tendency to a shorter survival time might thus have a selective

TABLE 17 Medically unattended deaths Call for medical assistance during last attack among patients with certain previous disorders (n=84)

	Total No.	Call for medical assistance No.	Per cent
Myocardial infarction	167	11	7
Angina pectoris	302	22	7
Heart failure	387	27	7
Hypertension	182	13	7
Diabetes	102	8	8
Suspect IHD symptoms	113	12	11
Other disorders	15	1	7
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It is seen from this table that there was no striking over or underrepresentation of any of these diagnoses with respect to attempts to seek medical assistance during the fatal attack, and the relative proportion of such attempts was not found to differ significantly in the presence or absence of any specific diagnosis.

COMMENTS

The criteria used for the various diagnoses in the present survey had to be set with regard to the *fact that with few exceptions the studied cases had* not been seen alive by the author For example the now usual criteria of acute myocardial infarction (typical history accompanied by characteristic electrocardiographic and biochemical abnormalities) could not be used in several cases in which the diagnosis was made without access to the necessary laboratory resources It is also probable that, in the event of a uniform clinical evaluation of the condition of the patients prior to death, some of the diagnoses of angina pectoris, heart failure and hypertension made in accordance with the used criteria would have been regarded as false positive. It appears probable, however that such an evaluation would ha e resulted in at least as many false negatives for these diagnoses Thus, of the autopsied cases in the present survey suspect symptoms —but not confirmed by a physician—suggestive of IHD were recorded for 13 per cent of the males and for 15 per cent of the females. No published results exist for comparison of the occurrence of previously diagnosed disorders in cases of death attributed to IHD occurring outside hospital, in which clear definitions had been given of these previous disorders.

The consistently higher prevalence of the recorded disorders in the non-autopsied cases would appear to be due to the fact that non-autopsied cases were to a greater extent under treatment for one of these disorders by the physician who issued the death certificate and thus this category could be expected to be "more ill than the autopsied cases. This will be dealt with later on in conjunction with the discussion of the validity of the IHD diagnosis (section F)

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The quoted study of Myerburg & Davis (1964) related solely to cases referred to the Medical Examiner and it may therefore be assumed that in this series the lower socio-economic groups were over represented This basis for selection may account for the relatively high proportion of cases with previous but not diagnosed symptoms suggestive of

E. POSTMORTEM FINDINGS

Varying autopsy rates have been reported from earlier community studies dealing with deaths attributed to IHD In the Belfast study (McNeilly & Pemberton, 1968) which covered cases occurring in hospital as well as medically unattended cases, the overall autopsy rate was 30 per cent From the Gothenburg study (Fodor 1969) an autopsy rate of 71 per cent for cases occurring outside hospital was reported In the present survey postmortems were made in 775 (80 per cent) of the 967 cases. The relatively high autopsy rate was considered to warrant an account of the postmortem findings

METHODS AND DEFINITIONS

The conditions for carrying out a postmortem were described in Part II (p 23) All postmortems except one were made at the *Government Institute for Forensic Medicine, Karolinska Institute* The one remaining patient had been treated with pacemaker and was autopsied at Serafimerlasarettet by police permission.

The procedure at the Institute included a gross examination of thoracic and abdominal organs In cases when a satisfactory explanation of the cause of death was not obtained in this examination, and in cases with a history of skull trauma or cerebral or cerebrovascular disease, the examination was extended to the skull contents. A complete postmortem was made also in cases when on the basis of the police investigation, the possibility of violent death could not be ruled out. In cases in which there was a suspicion of intoxication, appropriate specimens were also taken for postmortem chemical analysis

The routine examination of the heart included an inspection of the valves dissection of the coronary arteries with major branches, and slicing of the myocardium Microscopic examination was done only exceptionally—a microscopic examination of the heart in the present series only in 22 (3 per cent) of the cases.

According to the terminology employed at the Institute of Forensic Medicine a postmortem finding of myocardial infarction is classified as recent when the age of the infarction is judged to be less than one week (Lidholm, 1968) In all cases of myocardial rupture a recent infarction was considered to exist. In some of the cases in which the postmortem finding was classified as *coronary arteriosclerosis without mention of myocardial lesions* there was a report of coronary artery thrombosis but as the presence or absence of coronary thrombosis was not consistently reported, the frequency of this postmortem finding could not be analysed

In the reporting of postmortem findings these are indicated as mutually exclusive in accordance with the following principle on a finding of recent myocardial infarction a simultaneous finding of diffuse myocardial fibrosis is not recorded The same applies to a finding of isolated old myocardial infarction.

RESULTS

An account of postmortem findings by sex and age is presented in table 18 As will be seen, a *recent infarction was discovered in 145 (28 per cent) male cases and in 65 (26 per cent) female. This difference between the sexes is not significant* The same table shows that the commonest postmortem finding in both sexes was that of isolated old infarct, which occurred in nearly *half* of the cases (in 46 per cent of males and 40 per cent of females) a difference which is not significant. An autopsy finding of old infarct, isolated as well as associated with a recent infarct, existed in 63 per *cent of males and 54 per cent of females* ($p<0.05$) Diffuse myocardial fibrosis was found in somewhat higher—though not significantly different—proportion of female (21 per cent) than of male cases (16 per cent) as also was coronary arteriosclerosis without report of myocardial lesions, which was found in 10 per cent of male and 12 per cent of female cases.

No significant correlation to age existed for postmortem findings of recent or old infarction either in male or female cases. *Diffuse myocardial fibrosis was found in a significantly* ($p<0.01$) *larger proportion of the older male cases. A similar*

TABLE 18 Medically unattended deaths by sex, age and autopsy findings (Groups are mutually exclusive)

	Total		Recent infarct: Isolated		Recent infarct: With old infarct		Recent infarct: With myocardial rupture		Old infarct: Isolated		Diffuse myocardial fibrosis		Coronary arteriosclerosis without report of myocardial lesions	
	N	Per cent	No.	Per cent	No.	Per cent	No	Per cent	N	Per cent	No.	Per cent	No.	Per cent
MALES														
Age														
30—49	33	100	3	9	9	27	—	—	12	36	3	9	6	18
50—59	76	100	7	9	14	18	1	1	35	46	4	5	15	20
60—69	172[1]	100	12	7	24	14	7	4	87	51	24	14	18	10
70—79	172[2]	100	5	3	28	16	14	8	80	47	39	23	6	3
80—99	61	100	5	8	12	20	4	7	24	39	12	20	4	7
Total	514	100	32	6	87	17	26	5	238	46	82	16	49	10
FEMALES														
Age														
40—49	5		—	—	—	—	—	—	2		2		1	
50—59	16	100	—	—	3	19	—	—	4	25	3	19	6	38
60—69	70	100	8	11	10	14	3	4	29	41	9	13	11	16
70—79	110	100	9	8	16	15	6	5	49	44	24	22	7	6
80—99	51[1]	100	2	4	4	8	4	8	19	37	16	31	6	12
Total	252	100	19	8	33	13	13	5	102	40	54	21	31	12

[1] 2 cases excluded } Uncertain postmortem data.
[2] 3 cases excluded. }

TABLE 19 Medically unattended deaths by sex, autopsy finding and duration of last attack

	Duration (minutes)					
	0—15		16—			
	No.	Per cent	No.	p[1]	Unknown No.	Total No.
MALES						
Recent myocardial infarct	48	48	52	<0 01	45	145
Old myocardial infarct (isolated)	108	65	58	<0 05	72	38
Diffuse myocardial fibrosis	34	74	12	<0 05	36	82
Coronary arteriosclerosis without report of myocardial lesions	15	47	17	N S	17	49
Total subjects	205	60	139		170	514[3]
FEMALES						
Recent myocardial infarct	16	38	26	N S.	23	65
Old myocardial infarct (isolated)	28	60	19	N S.	55	102
Diffuse myocardial fibrosis	12	46	14	N S.	28	54
Coronary arteriosclerosis without report of myocardial lesions	9	43	12	N S	10	31
Total subjects	65	48	71		116	252[4]

[1] Of cases with known duration of last attack.

Denotes significance of difference between proportions at duration 0—15 and 16— minutes in presence and, respectively absence of the autopsy finding. N S = not significant (p>0 05)

[3] 5 cases excluded.
[4] 4 cases excluded.
} Uncertain postmortem data.

tendency existed among women, though not statistically significant.

An autopsy finding of coronary arteriosclerosis without report of myocardial lesions tended in both sexes to predominate among the younger age groups. For the men this age trend was significant ($p<0.001$) and for the women almost significant ($p<0.05$). Myocardial rupture with pericardial tamponade was found in 5 per cent of each sex. No case was reported with rupture of the interventricular septum. Myocardial rupture was more common in the higher age groups, and among men the age trend was almost significant ($p<0\ 05$).

A gross aneurysm of the left ventricle secondary to previous infarction was demonstrated by the pathologist in 18 cases (12 males and 6 females).

Autopsy findings in relation to duration of last attack

The duration of the fatal attack was estimated according to the principles set forth in Section A. Among the 519 autopsied males the duration of the last attack was known for 346 (67 per cent) and among females in 137 (54 per cent) of the 256 cases. There were, however, uncertain postmortem data in two of the male and one of the female cases with known duration of attack. There remained, accordingly 344 male and 136 female cases with classifiable postmortem findings and known duration of attack.

The result is shown in table 19, from which it is seen that, among men dying within 15 minutes of onset of symptoms of the fatal attack, postmortem findings of recent myocardial infarct existed in 48 cases (23 per cent) and among men with longer survival in 52 cases (37 per cent), a significant difference ($p<0.01$). An almost significant ($p<0\ 05$) tendency to shorter survival was recorded for the autopsy findings of isolated old myocardial infarct and diffuse myocardial fibrosis, whereas the presence of coronary arteriosclerosis without report of myocardial lesions or myocardial

TABLE 20 Medically unattended deaths by autopsy findings and prevalence of certain previous disorders. Males (n=319) (Groups mutually exclusive with respect to autopsy finding, whereas not with respect to previous disorder.)

	Recent myocardial infarct						Old infarct		Diffuse myocardial fibrosis		Coronary arteriosclerosis without report of myocardial lesions			
	Isolated		With old infarct		With myocardial rupture		Isolated						Unknown	Total subjects with same previous disorder
	No.	Per cent[1]	No	Per cent	No.	Per cent[1]	No.	Per cent[1]	No	Per cent[1]	No.	Per cent[1]	N	N
Myocardial infarction	1	3 1	28	32 30	1	4 1	57	24 61	4	5 4	1	2 1	2	94
Angina pectoris	4	13 3	30	34 23	1	4 1	76	32 58	14	17 11	4	8 3	3	132
Heart failure	7	22 4	31	36 19	3	12 2	86	36 52	30	37 18	4	8 2	3	164
Hypertension	5	9 5	10	11 17	2	8 3	33	14 56	7	9 12	4	8 7	—	59
Diabetes	4	13 8	8	9 17	—	— —	26	11 54	7	9 15	3	6 6	—	48
Suspect IHD symptoms	5	16 8	10	11 15	5	19 8	8	12 43	6	7 9	10	20 15	1	65
Other disorders	1	3 9	2	2 18	—	— —	3	1 27	3	4 27	2	4 18	—	11
None of abovementioned disorders	14	44 10	17	20 12	16	62 11	47	20 32	28	34 19	25	51 17	—	147
Unknown	—	— —	—	— —	—	— —	1		1		—	— —	—	2
Total subjects with same autopsy finding	32		87		26		238		82		49		5	

[1] Upper figure: per cent of total subjects with same autopsy finding.
Lower figure: per cent of total subjects with same previous disorder

TABLE 21 Medically unattended deaths by autopsy findings and prevalence of various previous disorders. Females (n=256) (Groups mutually exclusive with respect to autopsy findings, but not with respect to previous disorders)

	Recent myocardial infarct						Old infarct isolated		Diffuse myocardial fibrosis		Coronary arteriosclerosis without report of myocardial lesions		Unknown	Total subjects with same previous disorder
	Isolated		With old infarct		With myocardial rupture									
	No	Per cent[1]	No	Per cent	No	Per cent[1]	No	Per cent[1]	No	Per cent	No	Per cent[1]	No	No.
Myocardial infarction	1	5 / 3	5	15 / 15	—	— / —	23	23 / 70	2	4 / 6		6 / 6	—	33
Angina pectoris	2	11 / 3	12	36 / 18	4	31 / 6	30	29 / 44	11	70 / 16	8	26 / 12	1	68
Heart failure	5	26 / 3	15	45 / 15	5	38 / 5	50	49 / 51	15	28 / 15	8	26 / 8	1	99
Hypertension	4	21 / 7	7	21 / 12	3	23 / 5	26	25 / 44	11	20 / 19	8	26 / 14	—	59
Diabetes	4	21 / 13	6	18 / 20	5	38 / 17	9	9 / 30	5	9 / 17	1	3 / 3	—	30
Suspect IHD symptoms	4	21 / 11	5	15 / 15	1	8 / 3	18	18 / 47	10	19 / 26	—	— / —	—	38
Other disorders	—	— / —	1	3	—	— / —	—	— / —	—	— / —	1	3	—	2
None of abovementioned disorders	4	21 / 6	5	15 / 8	5	23 / 5	1	21 / 54	16	30 / 26	11	35 / 18	2	62
Unknown	1	5	—	— / —	—	— / —	—	— / —	—	— / —	—	— / —	—	1
Total subjects with same autopsy finding	19		33		15		102		54		31		4	

[1] Upper figure: per cent of total subjects with same autopsy finding.
Lower figure: per cent of total subjects with same previous disorder.

rupture was not associated with any significantly different duration of the fatal attack.

Among the women, as appears from table 19 none of the postmortem findings was associated with any significant difference of survival time.

Autopsy findings in relation to previous disorders

The criteria for the recorded disorders were the same as earlier reported in Section D As appears from these definitions, some of the recorded disorders are not mutually exclusive, so that the sum of disorders exceeds the sum of the corresponding number of cases. The result for the men is presented in table 20 and for women in table 21

A finding of a recent myocardial infarct (including myocardial rupture) among men did not exist in significantly different proportions in the presence or absence of any of the previous disorders recorded in table 20 Among the women the *same finding was significantly* ($p<0.01$) more common in the presence of diabetes of the 30 women with this previous diagnosis a recent infarct was found in half of the cases, while the same finding was made in 49 (22 per cent) of the 221 cases without diabetes in which the result of autopsy was known. In a separate analysis of myocardial rupture in relation to previous disorders, this finding existed for a significantly ($p<0.001$) larger proportion of the male cases with a negative than with a positive history of any of the disorders listed in table 20 (11 per cent and 3 per cent, respectively) No such tendency could be demonstrated for the females.

As appears from tables 20 and 21 a history of previous infarction was associated with a post mortem finding of old myocardial infarction in similar proportions of the two sexes in 92 per cent of men and 85 per cent of women. In the absence of a history of previous infarction an autopsy finding of old infarct was made for significantly ($p<0.001$) smaller proportions of each sex among males 57 per cent and among females 49 per cent. No significant difference in these proportions was obtained on comparison between the sexes

Likewise, among men with a history of angina pectoris, an old infarct was found in a greater proportion of cases (82 per cent) than in the absence of this diagnosis (57 per cent, $p<0.001$) A similar tendency was observed among women (63 per cent vs 51 per cent) but this was not significant. Comparison between the sexes revealed among *cases with a history of angina pectoris an old infarct* in a significantly ($p<0.01$) larger proportion of the males.

In the presence of a history of *heart failure* autopsy revealed an old infarct in a significantly ($p<0.01$) larger proportion of both sexes than in the absence of a history of this disorder (among males 73 per cent vs. 59 per cent and among females 66 per cent vs. 46 per cent) No significant difference between the sexes existed in this respect.

A finding of old myocardial infarction was made for not significantly different proportions of both sexes in the presence or absence of a history of hypertension, diabetes, suspect IHD symptoms or other disorders *Among the males with a negative* history of any of the disorders listed in table 20 a finding of an old infarct was made for 44 per cent as compared to 71 per cent in the presence of at least one of these previous disorders ($p<0.001$) No significant difference in this respect was found among women, nor in a comparison between the sexes.

In the presence of a history of previous infarction a finding of diffuse myocardial fibrosis was made for a significantly smaller proportion of the male cases and for an almost significantly smaller proportion of the female cases than in the absence of a history of this pervious disorder Among the *males with a history of previous infarction diffuse* myocardial fibrosis was demonstrated for 4 per cent (see table 20) and in the absence of a history of this diagnosis for 18 per cent (*$p<0.01$*) *As seen* in table 21 among the females the corresponding proportions were 6 per cent and 24 per cent, respectively ($p<0.05$) No significant difference in these proportions was found in a comparison between the sexes. For neither sex was the presence or absence of a history of any of the remaining disorders listed in tables 20 and 21 significantly differently associated with an autopsy finding of diffuse myocardial fibrosis

Of the males with a previous diagnosis of myocardial infarction a finding of coronary arteriosclerosis without report of myocardial lesions was made in one case (1 per cent) In the absence of such a history this autopsy finding was made for 48 (11 per cent) of the males ($p<0.01$) A similar tendency among males was found both for angina pectoris and heart failure. Of the cases with previous angina pectoris a finding of coronary arteriosclerosis without report of myocardial lesions was recorded in 4 (3 per cent) and of those with a history of heart failure also in 4 (2 per cent) In the absence of these disorders the corresponding proportions with this autopsy finding were, respectively 43 (12 per cent, $p<0.01$) and 45 (13 per cent, $p<0.001$) No significantly different proportion with this autopsy finding was found in the presence or absence of a history of the following disorders hypertension, diabetes, suspect IHD symptoms, and the category of other disorders in table 20 Of the 147 males with a negative history of the mentioned disorders a finding of coronary arteriosclerosis without report of myocardial lesions was made in 25 (17 per cent) but in 24 (7 per cent) of the 365 males with a history of at least one of these disorders and with classifiable autopsy findings ($p<0.001$) The corresponding proportion of female cases was found not to differ significantly in the presence or absence of any of the disorders recorded in table 21 with the exception of the group with suspect IHD symptoms In the latter group this autopsy finding was not demonstrated in a single case, as compared to 31 (13 per cent) of the 231 remaining females ($p<0.05$)

Of the 18 cases (12 male and 6 female) in which gross aneurysm of the left ventricle had been found on autopsy the aneurysm had, according to available reports, not in any case been diagnosed or suspected clinically For these cases a history was obtained of the following previous disorders myocardial infarction 9 (50 per cent) angina pectoris 8 (44 per cent) heart failure 11 (61 per cent) hypertension 1 (6 per cent) diabetes 2 (11 per cent) and suspect IHD symptoms 5 (28 per cent) For 2 cases (11 per cent) it was stated by relatives that the victim had not seen a doctor for years, and that there had been no signs of ill health prior to the fatal attack.

Three patients were treated with an artificial pacemaker According to the pathologist dislodgement of the electrodes or insulation defects could not be demonstrated at postportem inspection in any of these cases Postmortem analysis of the function of the impulse generator was undertaken in two cases, but significant electronic failure could be demonstrated in neither (Edhag, 1971)

COMMENTS

In evaluating the postmortem findings in this study the comparatively short interval between the onset of symptoms attributable to the fatal attack and death must be taken into consideration. As shown previously (Section A) of those for whom the duration of the last attack could be assessed, approximately half succumbed within 15 minutes. An infarct cannot be demonstrated by conventional methods until several hours after an acute ischaemic injury (Bjurulf *et al* 1967) Baroldi (1965) found that, even on careful examination of the heart, including microscopic examination of serial sections, a recent or acute coronary occlusion could not be demonstrated in over half of the cases of a series of sudden death attributed to IHD with previous clinical manifestations of this disease. The same author found an apparently normal myocardium in 31 per cent of his cases. Concurrent coronary occlusion and myocardial lesions in the same series were found in only 19 per cent of the cases. In a series of sudden death ascribed to IHD studied by Crawford (1963) using postmortem angrography and microscopic dissection of the coronary artery system, recent thrombotic occlusion was demonstrated in no more than 41 per cent of his cases.

From a postmortem series of sudden IHD death Adelson & Hoffman (1961) reported a recent coronary thrombus in one third of the cases. In the same study a myocardial scar was found in 55 per cent. Yater *et al* (1951) in their study of IHD deaths among military personnel, reported that a postmortem finding of recent infarction fell in frequency with rising age, while findings of

older infarctions increased with age. From the IHD Register in Gothenburg Fodor (1969) reported the finding of a recent myocardial infarction on autopsy among 39 per cent of deaths occurring outside hospital. In an earlier study of deaths attributed to IHD outside hospital in the Stockholm area (Wikland, 1968) postmortem examination carried out according to the same principles as in the present study revealed a recent myocardial infarct in slightly more than 20 per cent of the cases.

The male-female ratio for an autopsy finding of myocardial rupture in the present study was 1 1 Mitchell & Parish (1960) found, among deaths occurring within 24 hours of onset of the fatal symptoms of IHD that this ratio was 1 1.2. Crawford & Morris (1960) recorded a male excess with respect to this autopsy finding under the age of 70 whereas above this age the male-female ratio was reversed to 1 1 7 From a study dealing with hospitalized patients due to primary myocardial infarction Sievers *et al* (1961) reported an overall rate of myocardial rupture of 13 per cent for autopsied cases and a progressive increase with advancing age, but no significant sex difference. Although myocardial rupture in the present study was a less frequently made autopsy finding, the observations relating to distribution by age and sex in the two studies are in agreement. In a later study on a series of hospitalized patients with acute myocardial infarction Sievers (1963) recorded a male-female ratio for myocardial rupture of 1 1 4 He also recorded a healed infarction in 30—40 per cent of the fatal cases considered as victims of the first clinically manifest attack.

This remarkable discrepancy between postmortem findings of IHD and accepted clinical symptoms of this disease, as found both by Sivers (1963) and in the present study—in which an autopsy finding of old myocardial infarction was made for 60 per cent of the cases, whereas a history of previous infarction could be obtained for no more than 17 per cent— has also been reported by Bjurulf *et al* (1967) These authors found, in their epidemiological study from Malmö Sweden, that among cases in which a myocardial scar was demonstrated on postmortem examination 40 per cent had denied ever having had heart symptoms. Of those who had reported heart symptoms 74 per cent presented a typical history of either myocardial infarction or angina pectoris according to the definitions suggested by Rose (1962) and recommended by WHO (1963)

SUMMARY

Among the 967 medically unattended fatal cases of IHD autopsy had been carried out in 775 (80 per cent) A finding of a recent myocardial infarct was made for 28 per cent of the males and 26 per cent of the females. Myocardial rupture with pericardial tamponade was demonstrated for 5 per cent of each sex this finding was more frequent in the older cases.

A finding of old infarction was made for 63 per cent of the males and 54 per cent of the females. No significant age trend was recorded for this autopsy finding in either sex.

Among male cases a finding of diffuse myocardial fibrosis was made in 16 per cent and among females in 21 per cent. This autopsy finding was more frequent for the older cases of each sex.

An autopsy finding of coronary arteriosclerosis without report of myocardial lesions was made for 10 per cent of the males and 12 per cent of the females. This finding was more frequent for young subjects.

A recent infarct among males was demonstrated for a significantly larger proportion of those with a survival time exceeding 15 minutes than after a more rapid fatal attack. The reverse applied to an autopsy finding of isolated old infarct or diffuse myocardial fibrosis among males.

In the presence of a history of previous infarction a corresponding finding was made at autopsy for 92 per cent of the males and 85 per cent of the females A negative history in this respect was associated with an autopsy finding of old infarction for 57 per cent of the male and 49 per cent of the female cases.

F SOME ASPECTS ON THE VALIDITY OF THE DIAGNOSIS OF ISCHAEMIC HEART DISEASE ASSIGNED AS THE CAUSE OF DEATH

By validity is meant the extent to which a method measures what it purports to measure (Rose & Blackburn, 1968). From this point of view the most satisfactory criterion available was considered an autopsy finding in which, in the pathologist's opinion, sufficiently advanced IHD lesions existed for IHD to be accepted as cause of death in the absence of other more relevant findings.

Comparison between some categories of medically unattended deaths attributed to IHD

In the description of the criteria used in the present study (Part I) it was stated that a diagnosis of IHD when entered anywhere on Part I of the medical certificate of the cause of death qualified for entry. This procedure implies a departure from WHO instructions for classification of the underlying cause of death. In practice, however, this was seldom a problem as regards medically unattended deaths as in most of the death certificates, the pathologists nearly always recorded only a single diagnosis on this part of the certificate.

The acceptance of the pathologist's assessment of IHD as cause of death as the most satisfactory criterion from the point of view of the validity of diagnosis, however, implied that certain autopsy findings might possibly be considered to vary in significance of validity. Thus, a finding of a recent myocardial infarct might be thought to be more valid than a finding of other IHD lesions. Another factor which might strengthen the validity of the diagnosis is a history of previous infarction and angina pectoris. The following classification of deaths according to hypothetical degree of validity of the IHD diagnosis was adopted:

(1) Autopsy finding of a recent myocardial infarct
(2) Autopsy finding of other IHD lesions in cases *with* a history of previous myocardial infarction or angina pectoris
(3) Autopsy finding of other IHD lesions in cases *without* a history of previous myocardial infarction or angina pectoris and
(4) Non autopsied cases

It is hardly possible on the basis of the variables recorded in the present study to provide conclusive evidence regarding differences between these groups as to the validity of diagnosis. As previously shown in Section E, a recent infarct was demonstrated for a significantly larger proportion among those surviving longer than 15 minutes after the onset of symptoms attributable to the last attack. However, a rapidly fatal attack per se was considered to support rather than contradict the possibility of IHD as the cause of death.

Table 22 shows the distribution of the medically unattended deaths attributed to IHD by sex, age and basis of diagnosis. Within the same sex no significant difference was found between any of the three groups of autopsied cases as regards mean age. Among men the mean age, calculated from ten year age class means, was 67.5 years for autopsied cases compared with 70.7 years for the non-autopsied cases ($p<0.01$), among women 72.0 and 77.4 years respectively ($p<0.001$).

As shown in Section D, a comparison between non-autopsied and autopsied cases of both sexes revealed that in the former group the prevalence of a history of the following previous disorders was almost always higher, namely myocardial infarction, angina pectoris, heart failure, hypertension, and diabetes. A negative history with respect to any of these disorders among the non-autopsied cases was obtained for 9 per cent of the males and 11 per cent of the females. The latter cases appear primarily to represent the group in which the validity of the diagnosis of IHD as cause of death might be questioned.

Comparison of autopsy findings related to the heart between medically unattended deaths attributed to IHD and violent deaths

In an epidemiological survey from Malmö, Bjurulf *et al.* (1967) found in cases autopsied at an Institution of forensic medicine, and in which the cause of death was non-violent, that a finding of

TABLE 22. *Medically unattended deaths by sex, age and basis of diagnosis (7 males and 4 females not classifiable)*

	Autopsy findings				
	Recent myocardial infarct	Other IHD finding with previous infarction or angina pectoris	Other IHD finding without previous infarction or angina pectoris	Non-autopsied	Total
Age			MALES		
30—39	2	—	2	—	4
40—49	10	6	13	1	30
50—59	22	16	38	13	89
60—69	43	45	84	41	213
70—79	47	45	78	37	207
80—89	19	8	27	22	76
90—99	2	—	5	2	9
All	145	120	247	116	628
Per cent of all males	23.1	19.1	39.3	18.5	100.0
Mean age (years)	67.5	67.3	67.6	70.7	
Age			FEMALES		
40—49	—	2	3	—	5
50—59	3	3	10	3	19
60—69	21	19	30	11	81
70—79	31	3	56	27	137
80—89	10	15	23	31	79
90—99	—	—	3	4	7
All	65	62	125	76	328
Per cent of all females	19.8	18.9	38.1	23.2	100.0
Mean age (years)	71.9	71.9	72.1	77.4	

IHD was absent in 41 per cent. Among cases of violent death the same authors reported the absence of such a finding in 93 per cent. They considered that one explanation of this difference might be the usually younger age in the latter category.

To get a rough idea of the frequency of an autopsy finding of coronary arteriosclerosis sufficiently advanced to warrant the pathologist's judgement as severe among persons suffering violent death, of corresponding age and sex to those who died of IHD, a study was made of a sample of violent deaths examined by the same pathologists as assessed the medically unattended cases attributed to IHD. Violent deaths consisted of cases consecutively referred to the Government Institute for Forensic Medicine during a two-year period, the latter half of which coincides with the period for the IHD study. Comparison was made with consecutively referred cases attributed to IHD belonging to the present series.

The causes of violent death are presented in table 23. As will be seen, the majority of these cases were due to road accidents. Drivers of vehicles who, according to the police report, were responsible for the accident were excluded owing to the possibility that they may have been suffering from a predisposing diseased condition. In all of the cases in which the cause was given as jumping from high place the police report revealed suicide.

The following classification was used in respect of the pathologist's assessment of the lesions of the coronary artery system:

Moderate coronary arteriosclerosis: the coronary arteries including major branches possible to open. No total or near-total occlusion. This classification was adopted also in cases when, according to the

TABLE 23. Classification of coronary arteriosclerosis according to pathologists by sex, cause of death and age

	Degree of coronary arteriosclerosis			
	Violent deaths		Deaths attributed to IHD	
	Moderate or slight	Severe	Moderate or slight	Severe
		MALES[1]		
Age				
50—59	16	4	—	20
60—69	16	4	2	18
70—79	15	5	1	19
80—89	13	3	2	18
Total	60	16	5	75
		FEMALES[2]		
Age				
50—59	18	2	2	18
60—69	18	2	4	16
70—79	19	1	5	17
80—89	6	3	5	15
Total	61	8	14	66

[1] The causes of violent death were road accidents 69 cases shot by gun (2) jumping from high place (2) food obstruction of airway (1) drowning (1) and barbiturate intoxication (1)

[2] The causes of violent death were road accidents 56 cases stabbed by knife (1) jumping from high place (3) drowning (1) and barbiturate and/or carbon monoxide intoxication (8)

pathologist, the arteriosclerotic lesions in the coronary vessels were considered slight or non-existent.

Severe coronary arteriosclerosis at least one of the coronary arteries or one of the major branches totally or near totally occluded.

Table 23 shows for the deaths attributed to IHD and to violent causes, respectively the distribution by sex, age, and degree of coronary arteriosclerosis. As will be seen, there was certain overlap between the two categories in respect of the degree of coronary arteriosclerosis, but regarded as groups the difference is manifest. A comparison between corresponding ten-year age groups of the same sex in the respective categories showed that among victims of violent death the proportion of subjects with severe coronary arteriosclerosis is significantly ($p<0.001$) smaller than that of the corresponding group attributed to IHD. This significance of difference is valid for each ten year age group of both sexes except for the oldest females, among whom the number of violent deaths was too small.

SUMMARY

The best criterion available of IHD as the cause of death was considered to be an autopsy finding consistent with this diagnosis in the pathologist's opinion. The validity of the diagnosis was thought to be questionable primarily among those non-autopsied cases without a history of either previous myocardial infarction, angina pectoris, heart failure hypertension or diabetes. This group constituted 9 per cent of the male and 11 per cent of the female non-autopsied cases.

The severity of coronary arteriosclerosis was compared between two groups of consecutively examined cases. One group consisted of cases attributed to IHD the other of violent death in cases of corresponding sex and age. In both sexes and throughout all ages a significantly higher proportion of cases with severe coronary arteriosclerosis was found among those attributed to IHD.

Medically unattended deaths attributed to non-violent causes other than ischaemic heart disease

The object of this study was to attempt to illustrate the problem of medically unattended non-violent death from the following epidemiological aspects

(1) The approximate relation to ischaemic heart disease (IHD) of other non-violent causes of medically unattended death demonstrated in an autopsied material

(2) Prevalence of a history of previous myocardial infarction, angina pectoris heart failure hypertension, and diabetes and

(3) Autopsy findings related to the heart.

MATERIAL, METHODS, AND DEFINITIONS

The material consists of all cases autopsied at the Government Institute for Forensic Medicine, in which the following criteria were fulfilled

(a) death medically unattended according to the definition given in Part I (p 14)

(b) time and place of death according to the definitions in Part II (p 23)

(c) cause of death non-violent according to the pathologist, i.e. in the pathologist's opinion the cause was not an accident, poisoning or other violence according to ICD E-list (8th revision) and

(d) a diagnosis of IHD as specified on p 14 had not been entered on Part I of the medical certificate of the cause of death.

The death certificate was always issued by the pathologist concerned. In most cases only one diagnosis had been entered on Part I of the death certificate When more than one diagnosis had been entered on this part, the diagnosis recorded is that which according to WHO instructions shall be chosen as the underlying cause of death.

The definition for the abovementioned disorders agree with those given in Part II (p 37) These disorders were not recorded as mutually exclusive that is to say that, in case of concomitant prevalence of a history of more than one of these disorders in the same patient, all disorders were recorded as present.

The postmortem examinations were carried out by the same pathologists as examined the earlier reported medically unattended deaths attributed to IHD The procedure for the examination, as also the assessment of the postmortem findings, were the same as described earlier (p 49)

RESULTS

The series consists of 354 cases, of which 205 male (58 per cent) and 149 female (42 per cent) The distribution by sex, age, and cause of death is shown in table 24 Mean age at death, calculated from 5-year class means, was for males 58 7 years and for females 67 7 years In comparison with the mean age for corresponding autopsied cases attributed to IHD (for males 67 7 and for females 72.0 years) the difference is significant for both sexes ($p<0\ 001$)

As appears from table 24, the predominant cause of death in both sexes was diseases of the circulatory system, followed by diseases of the respiratory system. Among males the former group was represented by 99 cases (48 per cent) and among females by 89 (60 per cent, $p<0.05$) For diseases of the respiratory system the proportion of deaths among males was rather higher (24 per cent) than among females (14 per cent, $p<0.05$)

In table 25 is shown the distribution by sex and cause of death according to ICD B-list (8th revision) It is seen that B 46, all other diseases constitutes the predominant group in each sex. Among males in this group the cause of death in 12 cases (26 per cent) was a ruptured (non-syphilitic) aortic aneurysm and in 16 cases (35 per cent)

TABLE 24 Medically unattended deaths attributed to non-violent causes other than IHD. Distribution by sex, age and cause of death. (Numbers refer to International Classification of Diseases Detailed List 8th revision)

Age		All	Infective and parasitic diseases	Neoplasms	Endocrine, nutritional and metabolic diseases	Diseases of the nervous system and sense organs	Diseases of the circulatory system	Diseases of the respiratory system	Diseases of the digestive system	Diseases of the genito-urinary system	All other diseases
			ICD 000-136	ICD 140-239	ICD 240-279	ICD 320-389	ICD 390-458	ICD 460-519	ICD 520-577	ICD 580-629	
MALES											
0—9		4	—	1	—	1	1	1	—	—	—
10—19		1	—	—	—	—	—	—	1	—	—
20—29		3	—	—	—	1	2	—	—	—	—
30—39		13	—	—	2	1	3	3	—	1	1
40—49		29	3	—	1	—	11	9	4	—	1
50—59		47	1	1	—	—	24	9	11	1	—
60—69		61	1	3	—	—	50	16	9	2	—
70—79		34	1	2	—	—	20	7	4	—	—
80—89		12	—	1	—	—	6	3	2	—	—
90—99		1	—	—	—	—	—	1	—	—	—
	Total	205	6	8	3	3	99	49	31	1	2
FEMALES											
20—29		2	—	—	—	—	1	—	—	—	1
30—39		4	—	—	—	—	3	1	—		—
40—49		10	—	1	—	1	5	3	—	—	—
50—59		18	—	2	—	—	9	2	4	—	1
60—69		40	—	3	—	—	25	7	4	1	—
70—79		46	1	4	1	—	29	7	3		1
80—89		27	—	6	—	—	15	1	4	1	—
90—99		2	—	—	—	—	2	—	—	—	—
	Total	149	1	16	1	1	89	21	15	2	3

TABLE 25 Medically unattended deaths attributed to non-violent causes other than IHD by sex, cause of death according to ICD B-list (8th revision) and previous diagnoses and by sex, cause of death, and autopsy findings related to the heart

	All	Previous diagnoses					
		Myocardial infarction	Angina pectoris	Heart failure	Hypertension	Diabetes	Unknown
MALES							
B 5 Tuberculosis of respiratory system	6	—	—	—	—	—	—
B19 Malignant neoplasms	8	—	1	2	—	—	1
B21 Diabetes mellitus	3	—	—	—	—	3	—
B24 Meningitis	1	—	—	—	—	—	—
B26 Chronic rheumatic heart disease	11	1	—	5	—	—	—
B29 Other forms of heart disease	40	—	3	9	2	4	—
B30 Cerebrovascular disease	16	1	—	1	5	1	1
B31 Influenza	3	—	—	—	—	—	—
B32 Pneumonia	32	—	—	1	—	2	—
B33 Bronchitis, emphysema and asthma	8	—	1	2	—	—	—
B34 Peptic ulcer	12	—	1	—	—	—	—
B36 Intestinal obstruction and hernia	2	—	—	—	—	—	—
B37 Cirrhosis of liver	15	—	—	2	1	1	—
B39 Hyperplasia of prostate	1	—	—	—	—	—	—
B42 Congenital anomalies	1	—	—	1	—	—	—
B46 All other diseases	46	1	2	13	3	—	1
Total subjects	205	3	8	36	11	11	5
FEMALES							
B17 Syphilis and its sequelae	1	—	—	1	1	—	—
B19 Malignant neoplasms	16	—	—	4	—	1	1
B26 Chronic rheumatic heart disease	15	2	3	9	—	—	—
B27 Hypertensive disease	2	—	—	—	2	—	—
B29 Other forms of heart disease	20	—	2	11	4	3	—
B30 Cerebrovascular disease	20	—	—	2	3	—	1
B31 Influenza	1	—	1	1	—	—	—
B32 Pneumonia	14	—	1	1	—	2	—
B33 Bronchitis, emphysema and asthma	5	—	—	—	—	—	—
B34 Peptic ulcer	1	—	—	—	—	—	—
B36 Intestinal obstruction and hernia	1	—	—	—	—	—	—
B37 Cirrhosis of liver	6	—	—	1	1	—	—
B38 Nephritis and nephrosis	1	—	—	1	—	—	—
B45 Symptoms and ill-defined conditions	1	—	—	—	—	—	—
B46 All other diseases	45	—	—	—	—	—	1
Total subjects	149		7	31	11	6	5

pulmonary embolism, the figures for females being 16 (36 per cent) and 15 (32 per cent) respectively

The next largest group in table 25, B 29, was represented chiefly by the following Detailed List Nos. 426 (pulmonary heart disease), 428 (other myocardial insufficiency) and 429 (ill-defined heart disease). In that order the distribution among males was 10 (25 per cent), 14 (35 per cent) and

			Autopsy findings		
Old infarct					
Isolated	with recent infarct	Diffuse myocardial fibrosis	Coronary arteriosclerosis without report of myocardial lesions	Coronary or myocardial ischaemic lesions not demonstrated or insignificant according to pathologist	Uncertain post mortem data
			MALES		
—	—	—	2	2	2
—	—	2	3	3	—
—	—	—	—	3	—
—	—	—	—	1	—
3	—	1	1	6	—
4	—	1	11	3	1
1	—	—	3	10	2
—	—	—	—	3	—
—	—	4	8	20	—
—	—	—	2	6	—
2	—	1	4	5	—
—	—	1	—	1	—
—	—	1	3	11	—
—	—	—	—	1	—
—	—	—	—	1	—
5	1	4	13	19	4
13	1	15	50	115	9
			FEMALES		
—	—	—	1	—	—
—	—	4	2	8	2
2	—	2	1	10	—
—	—	—	1	1	—
1	—	4	2	13	—
1	—	5	3	12	1
1	—	—	—	—	—
2	—	—	2	9	1
—	—	—	1	4	—
—	—	—	—	1	—
—	—	—	—	1	—
—	—	—	1	3	—
—	—	—	—	—	1
—	—	—	—	1	—
5	1	3	11	24	1
12	1	16	25	89	6

10 (25 per cent) and among females, respectively 4 (20 per cent) 5 (25 per cent) and 7 (35 per cent)

The cerebrovascular disease group (B 30) consisted in one half (8) of male cases of subarachnoid haemorrhage and in 7 cases of intracerebral haemorrhage, the figures for females being 6 and 12 cases respectively

Of the relatively large group of pneumonias among males, lobar pneumonia was found in one half (16) of cases among females only in 2 out of 14 cases.

Prevalence of a history of some previous disorders

A record was made of a history of the following previously diagnosed disorders myocardial infarction, angina pectoris, heart failure, hypertension, and diabetes. The prevalence of a history of these disorders is shown in table 25 by sex and cause of death. It is seen from this table that a history of previous myocardial infarction could be obtained for no more than 1 per cent of either sex. A history of angina pectoris was obtained for 8 (4 per cent) of the males and 7 (5 per cent) of the females. A previous diagnosis of heart failure was recorded for 36 (18 per cent) of the male cases and 31 (21 per cent) of the female. Both for those with a history of heart failure and those with other disorders recorded in this table, the causes of death to which these cases were attributed were chiefly diseases of the circulatory system. When compared by sex none of the previous disorders recorded was found to be present in any significantly different proportion.

An assessment of the duration of complaints of continuous chest pain or dyspnoea prior to death was made on the basis of witnesses observations for cases attributed to any of the following causes chronic rheumatic heart disease, other forms of heart disease, and pulmonary embolism. Such information was obtained for 31 out of 67 (46 per cent) male and 26 (50 per cent) of the female cases. Death occurred within 15 minutes of onset of acute illness among 20 (65 per cent) of the males and 19 (73 per cent) of the females the sex differenc being not significant. Among the category of chronic rheumatic heart disease, altogether 12 of 16 cases of both sexes died within 15 minutes. In this category the cause of death had been specified as aortic stenosis in 10 cases, of whom 7 died within 15 minutes. Within this time interval there were 15 (71 per cent) out of 21 deaths attributed to other forms of heart disease. The corresponding number of deaths in pulmonary embolism was 12 out of 20 (60 per cent)

Autopsy findings related to the heart

Table 25 shows the autopsy findings related to the heart by sex and cause of death The sex proportions did not differ significantly for any of these findings. It appears from the table that a recent infarct was demonstrated in one case of each sex. In the male case with this autopsy finding death was caused by a ruptured aortic aneurysm, and in the female by pulmonary embolism.

As seen in table 25 an autopsy finding of old infarct was made in 16 (8 per cent) of the male cases and 13 (9 per cent) of female. Five of these deaths (3 males and 2 females) were attributed to chronic rheumatic heart disease and an equal number (4 males and 1 female) to other forms of heart disease. In the former group according to the pathologist, significant lesions of the aortic valves were present in all cases. In the latter group death was attributed to the following causes fatty degeneration of the myocardium (2 cases) pulmonary heart disease (1) arteriosclerotic narrowing of the mitral valve (1) and in one case myocardial scars which, in the absence of significant coronary arteriosclerosis, were judged by the pathologist to be possibly of rheumatic origin

COMMENTS

In the description in the earlier parts of this study of IHD deaths occurring outside hospital and other institutions for the chronically sick or aged the expression "medically unattended was preferred for this category The reason was that the commonly used designation for this category namely sudden death has no generally accepted definition as regards the time relation between onset of symptoms of the fatal attack and death. As regards deaths attributed to IHD an attempt was made to define this time interval in so far as this was considered feasible When we come to the medically unattended deaths dealt with in Part III the heterogeneity of this series must be kept in

mind However in a description of a similar series in the British or American literature, such cases would probably be characterized as sudden deaths The reason for this assumption is that, as mentioned earlier in addition to hospitals for emergency admissions the region studied is served by numerous public institutions devoted to the chronically ill or aged. Despite a relative shortage of vacancies at the latter type of institutions, it is very unusual that the care of patients in their terminal stage of a chronic disease takes place in their homes The small proportion of the recorded causes of death represented by malignant neoplasms in the present series must be viewed as a confirmation of this state of affairs. Therefore it appears appropriate to compare the findings in the present series with previously published studies referring to sudden non-violent death.

The fact that 80 per cent of all medically unattended deaths attributed to IHD occurring in the studied area were referred to the Government Institute for Forensic Medicine for postmortem examination suggested that a review of the medically unattended fatal cases attributed to other non-violent causes submitted to such examination, and occurring during the same period and in the same area as these IHD deaths, might provide a fairly good epidemiological picture of the spectrum of additional causes of medically unattended non-violent death. It was considered an advantage that the causes of death in this series were reported by the same pathologists as examined corresponding cases attributed to IHD as between-observer variations in the assessment were then avoided. In the light of the uniform assessment of the causes of death, it was considered to be beyond the author's competence to attempt to demonstrate falsely negative diagnoses of the causes of death from the IHD aspect

Kuller (1966) states in his extensive review of studies on sudden and non traumatic death in adults that, apart from IHD most other diseases associated with sudden death involve the circulatory system, and that haemorrhage following rupture of a blood vessel is perhaps the most common cause of sudden death after IHD Such haemorrhage, according to the same author may involve cerebral blood vessels in association with hypertension or in association with a developmental abnormality of the aorta or one of its branches, leading to rupture or dissection of an aneurysm. This assertion is confirmed by the findings in the present study indicating that 188 (53 per cent) of all deaths were attributed to diseases of the circulatory system. Of the cases in this category a ruptured aortic aneurysm or cerebral (including subarachnoid) haemorrhage was found on autopsy in nearly one third (61 cases) Simpson (1953) reporting on a British series of sudden death examined by the coroner found a subarachnoid haemorrhage in 4—5 per cent of the cases This proportion is in agreement with the figure of 4 per cent recorded for this condition in the present series. Secher Hansen (1964) in a large Danish series of deaths from subarachnoid haemorrhage, recorded that 93 per cent of the deaths occurred within 24 hours of manifestation of acute illness. A similar proportion of the 14 cases with this autopsy finding succumbed within this time interval in the present series thus 13 had been seen alive and apparently well within 24 hours prior to death.

Relatively scarce information is available in the literature on pulmonary emboli as cause of sudden death. According to Kuller (1966) most of the autopsy studies by the Medical Examiner have failed to report a high percentage of sudden deaths due to pulmonary emboli. However, as was pointed out by this author, it might be expected that in ambulatory patients with heart disease, especially in individuals under treatment for congestive heart failure or atrial fibrillation prior to death, pulmonary emboli may be a cause of sudden death. In the present study pulmonary emboli were demonstrated in 31 cases (9 per cent) Of these cases heart failure had been diagnosed prior to death for 12 for a further 4 cases there was a report of recent surgical trauma of the lower limb and in 2 cases a diagnosis had been made of a malignant neoplasm.

Rheumatic heart disease and particularly aortic stenosis is a widely recognized cause of sudden death (Bergeron *et al* 1954 Olesen & Warburg,

1958 Anderson, 1961) From various studies on the prognosis in aortic stenosis the incidence of sudden death has been reported as 10—25 per cent. As mentioned previously of the 10 cases attributed to aortic stenosis in the present study witnesses reports in 7 indicated that survival did not exceed 15 minutes after the onset of acute chest discomfort.

In a study of 33,265 autopsied cases from three Copenhagen hospitals, Nielsen (1961) found 58 cases of dissecting aortic aneurysm, 40 of which (69 per cent) had ruptured and caused death. A correct clinical diagnosis prior to death had been made in only 7 of 40 cases, 25 of which occurred within 48 hours of the onset of acute symptoms referable to the dissection. Of the 28 cases with ruptured aortic aneurysm in the present study reports of the duration of the acute symptoms referable to the aneurysm were found in 11 cases in 7 of these cases death occurred within 15 minutes of onset of these symptoms In none of the 28 cases was there a report that the diagnosis had been made or suspected prior to death. Nielsen, in his hospital series, found that the incidence of dissecting aortic aneurysm was 1 in 574 autopsies The same author (1961) in a series of forensic postmortems, found aortic aneurysm in only 1 of 1340 cases In the present series, including IHD deaths, dissecting aortic aneurysm with rupture occurred in 1 of 40 autopsies This considerable difference in comparison with the Danish series of forensic postmortems may be explained by the fact that the Danish material consisted of selected cases in which there was a suspicion of crime or other type of death requiring special investigation. Thus according to the quoted author in Copenhagen, with a population exceeding that of Stockholm, 2685 forensic postmortems were made during a 15-year period. The annual number of postmortem examinations carried out at The Government Institute for Forensic Medicine in Stockholm amounts to approximately 2500

As previously mentioned, among the men pneumonia was the second largest group of specific causes of death, in half of which a lobar pneumonia was demonstrated. Of the latter cases a report was obtained of advanced alcoholism in 14 (88 per cent) As cause of sudden death among alcoholics this disease does not appear to have attracted particular attention in the literature. Kuller (1966) suggests conceivable causes of sudden death among such patients, in the following order (1) pulmonary fat embolism from large fatty cysts in the liver (2) hypoglycaemia (3) alcoholic heart disease complicated by arrhythmia or pulmonary emboli (4) possible development of a rapidly fatal infection.

Owing to the predominant position of IHD among causes of medically unattended non-violent death it appears reasonable to assume that some of the cases in the present series attributed to other causes would have been attributed to IHD if an autopsy had not been done. The cases chiefly envisaged are those complying with the following criteria (a) age 50 years and on (b) symptoms of fatal episode resembling those in IHD and (c) the actual cause of death had not been previously diagnosed The causes of death associated with a last attack of symptoms definable in time and compatible with those recorded for IHD were chiefly chronic rheumatic heart disease, other forms of heart disease, and pulmonary embolism. Of 117 deaths assigned to any of these causes 73 conformed with the criteria set forth above as regards age and absence of a previous diagnosis of the fatal condition. Witnesses statement relating to the last attack were obtained for 35 patients of whom 24 (69 per cent) succumbed within 15 minutes of onset of symptoms associated with the acute attack. Assuming that a history of either previous myocardial infarction or angina pectoris represents specific clinical evidence of IHD among the corresponding autopsied cases aged 50 and above attributed to IHD such evidence could not be obtained for 530 whose survival time after the onset of the fatal attack was known for 310 164 of these (53 per cent) died within 15 minutes.

In the light of these findings it is tempting to make a theoretical assumption, although extremely tentative, concerning the proportion of deaths occurring within a recordable time interval following the onset of acute chest pain or dyspnoea that

might erroneously be attributed to IHD when occurring in the absence of a history of either previous myocardial infarction, angina pectoris or any of the three conditions other than IHD mentioned above, and without autopsy. Assuming that autopsy confirmation of the cause of death had not existed for any of the 345 cases in the present study aged 50 years and above, and with witness report of an episode of chest pain or dyspnoea defined in time and that all of these cases had been attributed to IHD some 10 per cent would be expected to have had lesions other than IHD sufficient to be considered as causative of death.

SUMMARY

A record was made of medically unattended deaths attributed to non violent causes other than IHD submitted to postmortem examination by the same pathologists as examined the corresponding cases attributed to IHD occurring within the same area and during the same period of time. The series includes 354 cases (205 males and 149 females). An analysis was made of age at death, causes of death, the prevalence of a history of previously diagnosed myocardial infarction, angina pectoris, heart failure, hypertension, and diabetes. A similar analysis was made with respect to autopsy findings related to the heart.

A comparison with corresponding IHD deaths showed a significantly lower mean age both for men and women in the present series. Despite the elimination of IHD the predominant cause of death in both sexes was diseases of the circulatory system, followed by diseases of the respiratory system. An account is given also of the more detailed categories of causes of death.

In comparison with corresponding IHD deaths the prevalence of a history of any of the following previously diagnosed disorders was found to be significantly lower: myocardial infarction, angina pectoris, heart failure, hypertension, and diabetes. Heart failure was the most common of the disorders recorded.

An old infarct was demonstrated on autopsy for 7 per cent of the males and 8 per cent of the females. A finding of diffuse myocardial fibrosis was made for 7 per cent of the males and 11 per cent of the females. Coronary arteriosclerosis without report of myocardial lesions was demonstrated for 24 per cent of the male cases and 17 per cent of the female.

Of all deaths at age 50 and above following an attack of chest pain or dyspnoea and not preceded by a history of a previous diagnosis of the fatal condition, when established by autopsy the cause of death was assigned to conditions other than IHD in 10 per cent of cases.

General discussion

When considering the problem of medically unattended deaths the question whether preventive measures are possible inevitably arises. As regards IHD it has been shown that life-threatening arrhythmic complications, which are especially common shortly after the onset of symptoms of the acute attack, are accessible to successful treatment (Geddes *et al.* 1967, Lawrie 1969, Stannard & Sloman, 1969, McNamee *et al.* 1970). The longer term prognosis for these cases is, according to the same authors, comparable to that for cases of uncomplicated acute myocardial infarction. As far as can be judged, the great majority of medically unattended IHD deaths in the present study succumbed to an arrhythmic complication of primary type, i.e. the complication was not preceded by signs of acute heart failure. In only 5 per cent of the autopsied cases was myocardial rupture with pericardial tamponade found. According to this reasoning the remaining cases would have died of theoretically reversible disturbance of the heart rhythm.

The prevention of IHD in individuals without clinical manifestations of this disease (primary prevention) would naturally be a radical and ideal solution of the entire problem. IHD is the result of a chronic disease with, as far as can be judged, a long incubation period from the start of the morphological disease process until the appearance of clinical manifestations, and the actual process is a result of a number of partially unknown interrelated factors (Biörck, 1968). Certain risk factors prior to the development of IHD have been identified in the Framingham study (Dawber & Kannel, 1966) among others, such as elevated serum lipid levels, elevated blood pressure, diabetes, lack of physical activity, excess body weight, the cigarette smoking habit, low vital capacity and gout. As shown in the Framingham study (Kannel *et al.* 1962) and later confirmed by Tibblin (1969) in previously asymptomatic individuals the incidence of myocardial infarction increases steeply with simultaneous occurrence of several risk factors.

It is still uncertain to what extent individuals clinically free from IHD symptoms can or will adhere to a programme of manipulation of risk factors during a lengthy period, and the experience hitherto from Gothenburg (Tibblin, 1971) is hardly encouraging. Biörck (1970) in an analysis of the consequences of a hypothetical elimination of all IHD mortality among the Swedish population from the age of 45 years and upwards, maintains that men would live on an average 4 years longer and women 3.5 years longer. In a corresponding conjecture concerning the elimination of all IHD mortality at ages between 45 and 65 years the same author found that the mean length of life for men would increase by only 1 year and for women by rather more than 6 months.

Thus as it would appear at present that the question of primary prevention of IHD in general is far from being assessable, the medically unattended deaths of this disease can even less be discussed from the aspect of primary prevention.

One of the questions which prompted this study was whether it might be warranted to establish a special mobile CCU of the type described from Belfast (Pantridge & Geddes, 1967, Adgey *et al.* 1969) and elsewhere. As shown by these authors, this is a practicable proposition for reducing the mortality outside hospital. The conditions for use of such a unit, however, differ in Belfast and the studied Stockholm area. In Belfast the movements of the unit are controlled from a centre situated within the same area as the central hospital, which also furnishes the extra staff needed for the unit. In the Stockholm area the ambulance organization is separate from the hospitals. Furthermore, in the

Stockholm area the reception of acute cases is distributed over a number of hospitals None of these hospitals is at present able to furnish the extra staff required for all-round-the-clock duty with such a unit. On the assumption that the necessary resources could be made available for a mobile CCU in the area, this could not—judging by the findings in this study—be expected to bring any significant reduction of the mortality outside hospital. As appeared from table 5 (p 26) of the cases with known duration of the acute terminal attack, 57 per cent died within 15 minutes. It appears hardly realistic to imagine that a mobile CCU would be of any meaningful assistance in such cases. As reported in section B of Part II, in the 257 cases with known duration of the last attack exceeding 15 minutes, an attempt to call for medical assistance had been made in only 82 (32 per cent) In these 82 cases constituting only 8 per cent of the total series, it might be of value to offer increased security during transit to hospital by having a mobile CCU Considering the extremely unfavourable time relation, for establishment of CCU care, between the onset of symptoms of the critical attack and death, it seems remarkable that, of these 82 cases in which assistance was summoned during the attack, 31 (38 per cent) died during transit to hospital. This suggests that the delay between call for medical assistance and the establishment of hospital care is comparatively short, which agrees with the experience from Belfast (McNeilly & Pemberton, 1968) Edinburgh (Oliver 1968) and Gothenburg (Lindström, 1969) In the cited studies it was found also that, in the sequence of events preceding hospital care for acute manifestations of IHD the greatest delay is in the interval between onset and calling for assistance From the Gothenburg study Lindström (1969) reported that, of 18 medically unattended fatal cases of IHD medical assistance had been sought immediately when symptoms appeared in only one. In the same study an attempt was made to analyse the causes of the delay in calling for assistance It was reported that in some cases the patients had probably tended to belittle their symptoms, and in others the cause was fear of hospitalization. Some patients, too had earlier had anginal pain and would not notice the difference between the pain to which they were accustomed and the symptoms in the fatal attack.

As a consequence of the findings in the Belfast study (McNeilly & Pemberton, 1968) it was suggested that, if the public were better informed about the significance of an attack of severe pain in the chest in middle-aged or elderly people, the time interval between the onset of symptoms and making the means of cardiac resuscitation fully available might be shortened. The same authors indicated the possibility of permitting selected patients, having survived one attack of infarction, and their relatives to call the ambulance service directly should another attack occur Fulton *et al* (1969) considered that, as the patients themselves are the cause of the greatest delay before admission to a CCU (Oliver, 1968) it would be theoretically possible to reduce this delay by educating the public to call their doctors or a mobile coronary service immediately they recognize their symptoms. However on more detailed examination this was seen to be fraught with difficulties patients often fail to appreciate the significance of their symptoms, and furthermore one would have to anticipate a large number of calls from patients who have misinterpreted other types of pain, the admission of whom would overwhelm already inadequate coronary care facilities

One might expect that patients with an experience of earlier myocardial infarction would tend to seek hospital care with less delay on the recurrence of symptoms than patients without such experience A perusal was made of the Serafimer lasarettet material of primarily CCU treated patients with clinically proven infarction during the period 1968—1969 This showed that, for patients with earlier clinically recognized *infarction*, the median time between onset and admission to the CCU was 3 hours 0 minutes. The corresponding median time for patients without earlier known myocardial infarction was 5 hours 5 minutes. In the former category of 138 admissions 84 (66 per cent) took place within 4 hours of onset of symptoms, in the latter 142 (49 per cent) of 278, an

almost significant difference ($p<0.05$) No significant difference was found between the proportions of patients with or without an earlier diagnosis of angina pectoris, heart failure, hypertension or diabetes, admitted within 4 hours of onset of symptoms.

The IHD Register in Gothenburg, however, revealed, according to Tibblin (1971) that patients with earlier known myocardial infarction do not tend to seek hospital care earlier than patients without this previous diagnosis. As far as is known, in none of these series were the patients with recurrent infarction consistently instructed concerning the importance of seeking hospital care without delay on the appearance of symptoms of a new infarction.

Since, as shown in the present study (table 5 p 26) in over half of the cases the acute symptoms in the medically unattended fatal cases did not last for longer than 15 minutes, the prospects of any radical reduction in the number of such deaths by systematic instruction appear limited From the observations made by witnesses concerning the symptoms in the critical attack in the present survey it was not possible to obtain more precise symptoms than acute chest pain and/or dyspnoea when the attack was of sufficient duration to be associated at all with any chest complaint.

The main reason why deaths in this study occurred outside hospital must be said to be the time factor in cases when the duration of symptoms of the acute attack did not exceed 15 minutes. Fulton *et al* (1969) found it difficult to conceive that, under any system, much can be done about patients dying within one hour of the onset of symptoms. This judgment must be considered in relation to the fact that, according to the same authors, the practice in Edinburgh is that patients first call their physician In the Serafimerlasarettet series from 1968—1969 of primarily CCU-treated patients with clinically demonstrated acute myocardial infarction, 13 per cent of the admissions took place within an hour of the onset of symptoms.

If on the basis of previous medical history one were to attempt to identify individuals in the risk zone for sudden or medically unattended IHD death, it appears natural to select primarily those individuals who have a positive history in respect of previous myocardial infarction and/or angina pectoris. As appeared from section D Part II of the 964 cases with known earlier history 167 (17 per cent) had a previously diagnosed myocardial infarction and another 202 (21 per cent) angina pectoris In accordance with the criteria for these diagnoses (p 37) some contact could in most cases have been expected to take place with a physician. Together these 369 patients represented 38 per cent of all 964 patients with known medical history Reports of the duration of attack in this category of cases were available for 242, of whom 153 (63 per cent) succumbed within 15 minutes of the onset of acute symptoms Judging from the results of this study accordingly the stated criteria for selection of cases with high risk of medically unattended IHD death would imply a considerable underestimate of the number of true candidates for medically unattended IHD death. Despite this, the relatively easily definable group of patients with earlier myocardial infarction and/or angina pectoris appears to be a suitable object for an attempt, by means of systematic information, to emphasize the importance of seeking CCU care as soon as possible e.g. on appearance of the following symptoms persistent (possibly nitroglycerine-resistant) pain in the chest or dyspnoea of more than 10 minutes duration.

Judging from the results of this study information of this kind could hardly be expected to bring any gain in the form of a reduced number of medically unattended IHD deaths if directed solely to the candidates for this fate. As appeared from Section B only in one case was there a report of a call for medical assistance by the victim in person. In the great majority of cases a call for medical assistance had been made by relatives or other witness, not seldom despite the patient's objections. Any information directed to patients with high risk of IHD death should then not be confined to the patients themselves, but extended to their relatives as well

It appears clear however that even with an opti

mal realization of the significance of acute IHD symptoms in identified patients at risk, the majority would probably die in their acute attack before adequate care could be offered. It seems to be generally agreed that in the majority of cases of early IHD death the causative mechanism is arrhythmia. A fatal outcome of a complication of other type such as myocardial rupture with pericardial tamponade was reported in only 5 per cent of the cases in this survey. The types of arrhythmias chiefly associated with a fatal outcome are of ventricular origin and of essentially two types, tachyarrhythmias and bradyarrhythmias.

Potentially fatal tachyarrhythmias in the form of ventricular fibrillation unassociated with cardiac failure or hypotension (primary ventricular fibrillation) has been reported to occur among 1—8 per cent of patients with acute myocardial infarction under CCU supervision (Killip & Kimball, 1967; Stock *et al.*, 1967; Lawrie *et al.* 1968; Lown & Vassaux, 1968; Stannard *et al.* 1969; Mogensen, 1970). This apparently low incidence of primary ventricular fibrillation would seem to be due to the fact that the CCU form of care implies an aggressive treatment of arrhythmias such as ventricular ectopic beats and ventricular tachycardia, which may precede the ventricular fibrillation. Mogensen (1970) found that the incidence of ventricular tachycardia observed during the first 12 hours in a CCU was 39 per cent for a delay of 0—3 hours between onset of symptoms and admission, thereafter significantly diminishing with increasing delay. Lawrie *et al.* (1968) reported that primary ventricular fibrillation occurred in 5.5 per cent of patients admitted to a CCU within less than four hours after onset of symptoms as contrasted with an incidence of 0.4 per cent when admission was delayed. An interesting observation made by the same authors is that, of the 12 patients who developed primary ventricular fibrillation and who had been under continuous ECG supervision for at least 15 minutes prior to the onset of the ventricular fibrillation, premonitory arrhythmias early enough to allow the institution of antiarrhythmic therapy were detected in only two. From the mobile CCU service in Belfast, Pantridge & Geddes (1967) reported that ventricular fibrillation was 25 times more common during the first 4 hours than during the following 24 hours.

In the presence of bradycardia Zoll *et al.* (1960) found that ventricular ectopic beats are particularly likely to trigger bouts of ventricular fibrillation. This clinical observation is supported by carefully controlled studies made in animals of the development of ventricular ectopic rhythms in the presence of slow driving rates (Han *et al.* 1966). The incidence of sinus bradycardia (rate 60/minute and less) in acute myocardial infarction has been reported on the basis of observations of CCU patients to be 11—19 per cent (Lown, 1966; Meltzer & Kitchell, 1966; Lawrie *et al.* 1967). A considerably higher incidence of bradycardia, however, has been reported from Belfast where, through the mobile CCU service, 73 per cent of patients with clinically proven acute myocardial infarction came under CCU care within 4 hours (Adgey *et al.* 1968). It was reported by these authors that 61 per cent of patients with posterior myocardial infarction initially examined within the first hour showed sinus or nodal bradycardia or atrioventricular block, or developed one of these bradyarrhythmias. It was also found that the incidence of bradyarrhythmia among patients with posterior infarction seen more than 4 hours after the onset of symptoms was markedly less than that among those seen earlier. The same types of bradyarrhythmia in the cited study were found to complicate anterior infarction in about half as many cases as posterior infarction.

Under CCU conditions it is well documented that, in acute myocardial infarction, both tachy- and bradyarrhythmia complications can to a large extent be mastered. In order to prevent tachyarrhythmia at an early stage of the acute attack Shillingford (1968) proposed routine administration of lignocaine in the patient's home by the general practitioner. In the light of their experience of the high incidence of early bradyarrhythmia, however, Adgey *et al.* (1968) warn against this procedure. Hellerstein (1969) suggested a trial study of a population of persons who have previously sustained a myocardial infarct or have documented angina pectoris, and who have ventri-

cular premature beats. He proposed that these patients should be given a syringe for self administration of atropine or lignocaine or similar drugs. However Oliver (1969) considered this approach premature. He pointed out that, in the first case, we do not yet know what to put in the syringe in the second, existing combinations of anti arrhythmic drugs are not uniformly effective or safe In view of the findings in the present study it appears hardly realistic that self administration, even of an effective anti arrhythmic substance or combinations of substances, could be expected to be of any value the duration of the symptoms was in most cases so short that the contents of the syringe after intramuscular injection would probably not have time to reach adequate blood concentrations. Also, judging from the findings in the present study in view of the apparent reluctance of the victim to make a phone call for medical assistance it would hardly appear realistic to expect so great an initiative on the part of patients as to perform a self-injection.

From the anti-arrhythmic point of view it would be desirable that, at the moment when the acute attack comes on, the patient already has an adequate blood concentration of a hypothetically active substance, in other words long-term prophylaxis. This presumes the identification of individuals running a risk of IHD death. Of the medically unattended deaths in the present series, myocardial infarction had been earlier diagnosed in 17 per cent of the cases and angina pectoris in another 21 per cent. Even if these patients constituted a minority in this series, they represent patients who have had some form of contact with a physician. As regards patients with previous myocardial infarction a high force of reccurence and mortality has also been shown, especially during the first year after the acute episode (Bjerkelund, 1957 Biörck, 1962 Paasikivi, 1970 Hofvendahl, 1971) These patients seem primarily to be of interest for future trials with medicamental prophylaxis against the arrhythmias which may be assumed to account for the majority of the medically unattended or sudden deaths in IHD If the substance is effective in this respect, its efficacy for patients who have had an acute myocardial infarction should be assessable after one year in a properly designed study

General summary

The object of this investigation was to study a) the medically unattended deaths from ischaemic heart disease (IHD) occurring in the Stockholm area during one year and b) the medically unattended deaths attributed to other non-violent causes occurring during the same period and in the same area, and in which a postmortem was made. For this purpose the survey was divided into three parts.

PART I This part presents the cases of IHD death in a defined population, that of Stockholm and some adjacent communes, and the relative proportions of these cases which were medically unattended which occurred in hospital, and which occurred in other institutions for the chronically ill or aged. Of the 1740 male deaths 39 3 per cent were medically unattended, 38.4 per cent occurred in hospital and 21 4 per cent in other institutions. Of the 1564 female deaths the proportions were virtually the reverse 21 7 per cent were medically unattended, 36.6 per cent occurred in hospital, and 41 2 per cent in other institutions In both sexes the mean age for the medically unattended deaths was lower than for the two other categories.

PART II An account is given in this part of the medically unattended deaths attributed to IHD comprising 967 cases (635 males and 332 females)

The time relationship between the onset of symptoms of the *fatal attack and death showed an extremely skew distribution among cases* for which such information was available, 61 per cent of the male and 48 per cent of the female cases succumbed within 15 minutes of onset of symptoms.

Reports of attempts to call for help during the acute attack were obtained in 9 per cent of cases. Only one patient personally sought medical assistance.

The majority of deaths occurred at home Of men below 67 years (the general pensionable age) 9 per cent died at work. In most cases the acute onset occurred during rest.

A history of previous myocardial infarction was obtained for 20 per cent of the males and 12 per cent of the females, angina pectoris in 32 per cent of the male and 30 per cent of the female cases, heart failure for 38 per cent of the males and 44 per cent of the females hypertension in 14 per cent of the male and 28 per cent of the female cases. The prevalence of a history of diabetes among the men was 9 per cent and among the women 13 per cent. For a further 11 per cent of the males and 13 per cent of the females, symptoms suggestive of IHD which had not been confirmed by a physician were reported, and in another 2 per cent of the entire series death was preceded by disorders such as hyperlipaemia, gout or chronic respiratory disease A negative history with respect to any of the abovementioned disorders was recorded for 24 per cent of the males and for 20 per cent of the females

A diagnosis of previous myocardial infarction was more frequent among younger individuals of both sexes. The reverse was found for the prevalence of a history of heart failure

The presence of angina pectoris among the males was associated with shorter survival, as was heart *failure in female cases*

Postmortem examination was carried out in 80 per cent of the cases. A finding of recent myocardial infarct existed for 28 per cent of the males and 26 per cent of the females Myocardial rupture with pericardial tamponade was demonstrated in 5 per cent of each sex this finding was more frequent in the more elderly cases. A finding of old infarction was made for 63 per cent of the males and 54 per cent of the females. No significant age trend was recorded for this finding in either sex. Among

male cases a finding of diffuse myocardial fibrosis was made in 16 per cent and among females in 21 per cent thus finding was more frequent for the more elderly cases of each sex. A history of previous infarction was associated with a postmortem finding of old myocardial infarction in 92 per cent of the males and 85 per cent of the females A negative history in this respect in conjunction with an autopsy finding of old infarction existed in 57 per cent of the male and 49 per cent of the female cases

PART III An account is given in this part of the medically unattended fatal cases attributed to non violent causes other than IHD occurring within the same area and during the same period as the corresponding IHD deaths, and examined by the same pathologists. This series comprises 354 cases (205 males and 149 females) Despite the elimination of IHD the predominant cause of death in both sexes was diseases of the circulatory system, followed by diseases of the respiratory system.

Including IHD of all deaths at age 50 and above following an attack of chest pain or dyspnoea and not preceded by a history of a previous diagnosis of the fatal condition when established by autopsy the cause of death was assigned to conditions other than IHD in 10 per cent of cases.

GENERAL DISCUSSION Here the medically unattended IHD deaths are considered from various conceivable preventive aspects.

Apart from the considerable organizational problems the provision of a special ambulance service with personnel and equipment for cardiac resuscitation could hardly under present conditions, bring more than marginal gains in terms of saved lives. This conclusion is based on the following observations

a. the extremely unfavourable time relation between the acute onset and death, and

b the remarkable delay between the acute onset and measures taken to seek medical assistance in cases when the duration of the attack should have made such measures possible.

It is, of course, very urgent to inform patients of the importance of quickly seeking adequate care on the occurence of symptoms of acute myocardial infarction. Until the load on existing coronary care units under such circumstances can be evaluated, however instructions should initially be confined to known high-risk patients and also to their relatives.

It appears clear, however, that even with an optimal realization of the significance of acute IHD symptoms in identified patients at risk, the majority would probably die in their acute attack before adequate care could be offered. As far as can be judged, in the great majority of the very early cases of death, arrhythmia is the responsible mechanism. On this assumption attempts at medicamental arrhythmia prophylaxis appear to be extremely urgent. It would be desirable that, at the moment when the acute attack comes on, the patient already has an adequate blood concentration of a hypothetically active substance. This could only be accomplished by a long term prophylactic regimen in identified individuals at risk. Initial trials with arrhythmia prophylaxis are advocated in patients recovering from an acute myocardial infarction, since the risk of fatal recurrence is known to be particularly high during the first year after the acute episode. Consequently the efficacy of the substance used for this purpose should be assessable after one year provided that these trials are properly designed

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This Round-The Table Conference aimed at assembling some European scientists who had significantly contributed to the rapidly developing field of platelet aggregation and at allowing these workers to discuss a number of open questions. These discussions were extremely free which is somewhat apparent from the fact that not all topics have received equal coverage Before the meeting was opened a minute of silence was maintained by all participants in pious remembrance of their eminent colleague Dr Shirley A. Johnson who suddenly passed away on September 11th, 1970

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INTRODUCTION

CURRENT CONCEPTS ON
NORMAL AND MODIFIED PLATELET AGGREGATION

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There are two reasons for including in this brief introduction some historical remarks first it is to be hoped that our discussions later on will be restricted to the latest developments in the field, and that therefore older work will not be mentioned in any detail secondly because a study of the earlier literature shows that quite a few of the major problems which still are of primary importance today have evolved already years, if not decades, ago

Platelet aggregation be it *in vivo* after injury of the vascular endothelium, or *in vitro* in connection with blood coagulation, is a very striking phenomenon and therefore it is not astonishing that already in the last century hematologists were aware of it and developed distinct ideas as to its importance under physiological and pathological conditions. This era which remains linked to the names of Bizzozero Eberth and Schimmelbusch and Hayem, to mention but a few was followed by decades of almost complete disregard for the platelets. This dark episode was lit by a remarkable article by Wright and Minot (27) who in 1917 described in great detail irreversible aggregation which they found linked to spontaneous contraction of the primarily loose platelet masses formed *in vitro* they also came very close to describing thrombin as the essential agent capable of inducing "viscous metamorphosis"

Twenty years later Apitz (2) again was intrigued by platelet aggregation. He thought that it resulted from the micro-coagulation of fibrinogen, whereby a non-fibrous, sticky modification of fibrin was formed. Even earlier Roskam (19) had described the presence on the platelet surface of fibrinogen thus the idea that the fibrinogen-fibrin transition on the platelet surface might be of importance seemed quite justified In the light of much more recent work to mention only the findings of Solum (23) the participation of fibrin precursors in the introduction of profound platelet changes which are linked to aggregation, deserves renewed interest

Around 1960 however it had become clear that thrombin was not the only inducer of platelet aggregation The work of Hugues and Bounameaux in particular showed that inorganic particles, opsonized yeast and, most important, the collagen component of connective tissue lead to platelet adhesion and aggregation culminating in "viscous metamorphosis" (see review by Roskam et al. 20) In this context is of some importance to note that very recently a second component of connective tissue besides collagen, has been described, to which platelets seem to adhere without undergoing far-reaching alterations (24)

Several other important discoveries were

made at about the same time the dependence of platelet activities on an intact carbohydrate metabolism (3) the isolation and description of a contractile protein resembling actomyosin in platelets (4) and finally the recognition that adenosine 5'-diphosphate (ADP) is a potent inducer of platelet aggregation (6) This latter finding, which originally was based on the liberation of aggregating material from red cells (factor R of Hellem, 7) got its full significance only with the observation that platelets themselves, under the influence of a suitable inducing agent such as for instance thrombin, release enough of the dinucleotide to induce their own aggregation. The recognition of this phenomenon remains a most important step in the understanding of the mechanism of platelet aggregation which accordingly appears as a self perpetuating process.

Thus, almost ten years ago, the intimate linkage of the release reaction to platelet activity was recognized. Simultaneously Parmeggiani (17) was first to demonstrate the disappearance of the typical, osmiophilic storage organelles within the platelets under the influence of thrombin This obviously correlates with the appearance in the surrounding medium of serotonin adrenaline adenine nucleotides, platelet factor 4 potassium, and calcium under the same conditions (9)

The so-called inducers of platelet aggregation deserve a more detailed discussion which will be limited to those agents or closely related materials which play a direct role under physiological or pathological condition The first class to be mentioned are proteases with thrombin as their most important representative In order to show activity these enzymes must be of the trypsin type with respect to specificity chymotrypsin for instance is quite inert towards platelets Even within the trypsin-like agents there are as shown first by Davey and Luscher (5) striking differences neither the enzymes from the venom of *Ankistrodon rhodostoma* ("Arvin") or of *Bothrops atrox* ("Reptilase") nor staphylocoagulase-thrombin although all of them capable of clotting fibrinogen show any effect on platelets they induce neither aggregation nor release It has been concluded from these findings,that the fibrinogen-fibrin transformation is of no importance for the induction of platelet aggregation On the other hand it remains an interesting fact that all these enzymes devoid of activity towards platelets remove only the A-peptides from fibrinogen Thus, if the action of proteases on platelets was mediated through intermediates of the fibrinogen-fibrin transformation it obviously must be products devoid of both peptides A and B which are active The effect of the enzyme from *Ancistrodon contortrix contortrix venom*, which preferentially releases peptide B (8) unfortunately has not yet been tested as to its effect on platelets. In view of the work of Solum (23) on the induction of aggregation by fibrin-precursors, and based on the pronounced activity of fibrin monomer complexes as demonstrated recently again by Larrieu et al (10) such a reaction mechanism for proteases seems not so far fetched It should be noted, however that thrombin still aggregates platelets which have been excessively washed and even pretreated with chymotrypsin in order to remove adhering fibrinogen Thus, at present, the existence of a specific alternative substrate perhaps a membrane constituent proper still seems a more likely explanation for the mode of action of aggregation-inducing proteases. Such a thrombin-labile membrane constituent has recently been described by Majerus and Baenziger (1?)

To the second group of inducing agents belong materials such as collagen and immune complexes, i.e. antigen-antibody complexes, aggregated gamma-globulins, and γ-globulin-coated particles (14 15) These are relatively bulky materials devoid of enzymatic activity Like thrombin they give rise to a release reaction even in the absence of calcium ions If these are present aggregation and manifestation of contractile activity in the aggregates ensue

The third class of inducers is represented by low-molecular substances, to mention ADP in the first place followed by adrenaline noradrenaline and serotonin Added to platelets in small amounts, and in the presence of calcium

ions and of fibrinogen ADP aggregates platelets reversibly above a certain threshold concentration these aggregates become irreversible and a release reaction occurs. The endproduct of this second phase aggregation is the same as the one obtained under the influence of agents of the thrombin – or collagen – types of inducers.

With this evidence available the stage was set for a most plausible theory of aggregation any class of inducer gives rise to a release reaction and the ADP thus made available is then responsible for the onset of aggregation and its secondary manifestations, i.e. morphological and biochemical changes and activation of the contractile system. Accordingly several hypotheses for the mode of action of ADP on platelets have been proposed most of them are based on the concept of the inhibition by the dinucleotide of an "ecto-ATPase" localized on the platelet membrane (11)

Such a theory implies that ADP added in adequate amounts, should exert a profound influence on each individual platelet in particular it should lead to a release reaction and availability of platelet factor 3 Unfortunately this is not the case if aggregation is prevented e.g. simply by omitting stirring of the platelet suspension no release reaction is observed (28). This is quite different for agents such as proteases, collagen or immune complexes these will induce release without aggregation As discussed above the proteases may well be a special case collagen and immune complexes on the other hand may perhaps be described as highly specific surfaces to which platelets adhere and subsequently undergo alterations culminating in the release reaction. This principle of the contact with a specific surface as the trigger event for the induction of second phase aggregation perhaps is quite generally applicable For the case of ADP induced aggregation it then might imply that the contact between the platelets was in fact the trigger event leading to release and second phase" aggregation. This would make it understandable then that prevention of aggregation abolishes the effect even of large amounts of ADP on platelets. This hypothesis on the induction of release by cell contact can be tested. O Brien (16) already some time ago became aware of the fact that propinquity of the platelets, as brought about by mild centrifugation may lead to the symptoms of second phase" aggregation. Recently this phenomenon has been studied again by Massini and Luscher (13) They found that centrifugation of platelets at 37°C induces the release of serotonin and adenine nucleotides in amounts comparable to those obtained with collagen or ADP This reaction has a pH optimum of about 8 it is independent of cofactors such as Ca^{++} and fibrinogen. Obviously platelets must first be conditioned before they are capable of acting as inducing surfaces. In the cited *in vitro* experiments, this is done by working at an elevated pH. Under physiological conditions they most likely undergo a specific type of conditioning, which probably involves ADP in a hitherto not clearly defined way It should be noted that this is still quite hypothetical and comments to these ideas are most welcome

Up to now this discussion has dealt with normal aggregation and it seems appropriate to terminate with a few remarks on the modified process. This modification of platelet aggregation consists mainly in its inhibition The interference with aggregation not only is of considerable practical importance as an alternative possibility for the prophylaxis of thrombosis, but also of theoretical interest because of the insights into the active process gained by studying the nature and reaction mechanism of its inhibitors.

The earliest thoroughly studied inhibitors were adenosine and its analogues. Their mode of action originally believed to consist in a competition with ADP for receptors, seems to be more complex and will be discussed in detail later in this symposium. Starting out with the observation that antiadrenergic drugs in vitro may depress successfully second phase aggregation (11) the effects of many more drugs were investigated. A wide variety of agents with widely differing pharmacological properties was found to be active Among them are tranquil-

izers, muscle relaxants, antiinflammatory agents, and others (for review see 11) Aspirin ought to be mentioned particularly since it may exert its inhibitory effect by blocking, perhaps by acetylation essential sites on the platelet surface (1) it also inhibits the release reaction brought about by "propinquity i.e cell contact. It is particularly noteworthy that blocking of free amino groups in collagen and in immune complexes also abolished their inducing properties (26) this suggests that a specific pattern of charge distribution is required for the induction of aggregation and release by contact" in general

One inhibitor in particular has opened a new outlook on the process of aggregation prostaglandin PGE_1 This interesting material is a powerful stimulator of adenylic cyclase (25) and thus is involved in the regulation of the intracellular level of cyclic adenosine 3,5 monophosphate (c AMP) Further work has shown that there seems to exist a remarkable correlation between platelet aggregation and c AMP levels (22) inducers generally depress it, whereas many inhibitors lead to its increase As shown by Salzman (21) this effect may be mediated as well through adenylic cyclase as trough phosphodiesterase the enzyme involved in c AMP breakdown How to explain this obviously close relationship between aggregation and c AMP? We know that there is no link to mobilization of glycogen or to carbohydrate metabolism in general, as observed in other cells and an alternative pathway has not yet been proposed It is perhaps allowed to draw the attention to the finding, that in sarcoplasmic reticulum, c AMP seems involved in a pump system controling the intracellular calcium level (18) and thereby the contractility of the muscle fiber In view of the fact that platelets contain remarkably high amounts of contractile material it seems reasonable to postulate the existence of a comparable system for platelets, i e a c AMP-dependent "relaxing factor" There can be little doubt that calcium-ions are essential for many steps in the sequence of platelet activities, and therefore a system controling their intracellular availability would be expected to play a central role This again is a hypothesis which is open to discussion.

The last years have been characterized by an ever increasing effort to gain more insight into the mechanism of platelet aggregation, which in relation to hemostasis, thrombosis, and intravascular activation of the clotting system remains a most important and urgent problem. Most encouraging results have been obtained, but at the same time it has become more and more obvious, how complex the problem is. It is a long way from Apitz s assumption of a passive trapping of platelets in a sticky modification of fibrin to the picture as it evolves today which includes all the manyfold activities of a living cell In what consist the primary effects of the many different types of inducers on the platelet? What system is responsible for the transformation of different stimuli into a uniform pattern of sequential reactions? It is quite clear that the solutions to these and many other problems are intimately linked to the problem of membrane structure and reactivity in general, to the linkage of energy metabolism to transport and intracellular structure Work on platelet aggregation therefore has become part of a very general problem, i.e the reactivity of any living cell to external stimuli. That we are dealing with a particularly active and highly specialized cell does not simplify the problem. Its solution certainly will come from a cooperative effort and from an open-minded exchange of ideas between specialists in many different fields.

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Question N 1

A. WHICH HYPOTHESIS ON PLATELET AGGREGATION BY ADP WOULD SEEM TO BE THE MOST PLAUSIBLE ?

B ARE COFACTORS OF PROTEIN NATURE INVOLVED IN NORMAL PLATELET AGGREGATION ?

1 G V R BORN Platelet Aggregation in Physiological Systems
2. K. BREDDIN and H J KRZYWANEK Plasma Factors for Platelet Aggregation
3 J VERMYLEN M B DONATI and G de GAETANO Protein Requirement for Platelet Aggregation

DISCUSSION

J HUGUES
G V R BORN
E.F LUSCHER
S BYGDEMAN
J R. O'BRIEN
R. GROSS
A. SHARP
J CAEN
R M HARDISTY
M.J LARRIEU
H REUTER
J VERMYLEN
A.S. DOUGLAS
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1 PLATELET AGGREGATION IN PHYSIOLOGICAL SYSTEMS

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One of my sisters who is not a doctor learned something about platelets a few days ago whereupon she wrote the following poem

A thrombocyte said to his mate
Come on, let s start to aggregate
Clinicians always call us clot
But we know better   we are not !
Not tc be understood is our fate ...

Nicola Begent and I (1) have recently started with a new method to try to see how platelets aggregate *in vivo* We apply ADP by microiontophoresis though micropipettes to the small veins or arteries in the cheek pouch of the anaesthetized hamster The tip of the pipette is 1 or ? microns in diameter and it is filled with 10 mM ADP We have measured the rate of ADP coming out by using very high sepcific radioactive ADP   it is of the order of 10^{-4} mole ADP/sec. A very small current is used   this is important   the current is a thousand times less than the current that people use to damage a vessel Usually we use 100-300 nA but it works even with less than 100 nA. A few seconds after the current begins to pass, a white body i.e. a platelet aggregate forms in the vein opposite the pipette tip We analyse the process by filming the site and measuring the rate of growth of these white bodies. Our idea is to analyse this system from every single point of view The possibility of damage is assessed by serial electron micrographs. I shall summarize our findings. In the endothelial cell, the only abnormality is pallor i.e. diminished osmophility of the cytoplasm   what that means we do not yet know The surfaces are intact, the cell is not swollen and the junctions between the cells are normal. Thus is apparently the first time in which it has been shown that platelets can adhere by whatever means, to what looks like almost normal endothelium. We quantitate the growth of each white body by measuring the height and the base and we assume – which is of course not a true but a reasonably close assumption – that this white body approximates a segment of a sphere We calculate the increase in volume and surface area.

We have found that if the volume of the white body in cubic microns is plotted against time in seconds, the result is a curve If you plot the increase in volume semi-logarithmically against time you get a straight line indicating that there is a first order rate constant for the growth of white bodies. We are investigating the influence of a variety of parameters on this first order rate constant to see how it is affected by variables such as concentration of ADP of fibrinogen of calcium, etc.

Hugues (3) showed many years ago that such platelet thrombi grow very quickly *in vivo*   the question is, how does it happen ? When one

thinks about the *in vitro* results, one of the crucial questions is whether the specific release reaction really plays a part in vivo as physiological phenomenon. The answer is not yet known. If sufficient thrombin is added to platelets *in vitro* the release reaction can be very rapid. It is a matter of seconds rather than of minutes. On the other hand the phenomenon *in vivo* is perhaps a matter of milliseconds rather than of seconds. If ADP plays a part in this at all then the specific release reaction, that is release of nucleotide and serotonin from the storage granules, seems to me to be unlikely as the primary event. I expressed the same thing already some years ago but then it was just a guess; now there is at least some evidence.

What can the primary event be? Before we come to that, I would like to make a very clear distinction between two kinds of platelet aggregation, one of which one might call artificial and the other physiological. I think confusion has often arisen by people not distinguishing clearly enough between artificial systems and what is happening in the blood stream; the latter is what Begent and I are trying to investigate. In artificial systems many different things can aggregate platelets, e.g. positively charged long molecules as the removal of negative charges by neuraminidase. The question is how, if at all, this is related to what happens physiologically.

To get closer to the physiological system we have also been investigating the rapid shape change which is the first event when platelets are exposed to ADP and to most other aggregating agents ( ). An optical method used for measuring platelet aggregation was adapted for measurements of the change in shape of platelets that rapidly follows the addition of the aggregating agent ADP. Measurements were made using dilute suspension of platelets with sufficient EDTA to prevent their aggregation. Measurements of the velocity and magnitude of the optical effects of the shape change were highly reproducible. Volumetric measurements showed that the shape change is not associated with an increase in mean platelet volume. The velocity of the shape change had a temperature coefficient of about 4.5. The velocity and magnitude of the shape change were not affected by pH between 5.8 and 9.2. The dependence of the velocity of the shape change on the ADP concentration was in accordance with Michaelis-Menten kinetics. The K_m was about 7.2×10^{-7} M. The velocity of the shape change was inhibited competitively by ATP, adenosine and 2-chloroadenosine but not by AMP. When the inhibitors were added after the maximum of the shape change they caused a concentration-dependent diminution in the record of the change. The results suggest that the shape change is initiated by reaction of the agonist ADP with specific receptor sites on the platelet membrane which leads to energy-requiring changes in the structures responsible for maintaining the disk shape of normal platelets. These observations raise a number of important questions. The evidence is that ADP does not penetrate the cell membrane, nor does ATP. In the presence of a high concentration of EDTA an enzymic reaction on the platelet surface is very unlikely. Therefore the shape change is presumably caused by a reaction of the nucleotide with a receptor on specific chemical grouping in the platelet membrane.

The other very interesting thing about the rapid shape change is the remarkably high temperature coefficient. Most enzyme reactions have a Q_{10} of 2.5 or so, whereas that of the shape change is twice as high. Therefore the shape change depends very markedly on temperature. What can this mean? The temperature coefficient of muscle contraction is only about 1.5, that is with different muscles all the way from the sloth to the cat. Temperature coefficient for reactions of whole cells cannot be interpreted but a high coefficient suggests one or more processes with high activation energies. The most plausible is the depolimerization of a highly organised protein structure; one immediately thinks of microtubules or of some component of the membrane or both. This is compatible with electron microscopic evidence of Hovig and Bang, and others.

Is the rapid shape change necessary for

physiological aggregation? We hope to be able to answer this soon. If the shape change occurs physiologically how is it produced? I think the evidence suggests that some membrane ATP is broken down to ADP in a reaction which causes the initial conformational change in the membrane.

I have left several loose ends, and we are going to talk about cyclic AMP and calcium later.

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2 PLASMA FACTORS FOR PLATELET AGGREGATION

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With the Platelet Aggregation Test (PAT) we have been able to demonstrate an enhanced platelet aggregation in the platelet-rich plasma of patients with thrombosis, peripheral atherosclerosis, diabetes or recent myocardial infarction (1-4). The PAT allows the evaluation of the aggregating capacity of platelet-rich plasma without addition of any aggregating substances.

Performance of the PAT

An aliquot of 1.5 ml of platelet-rich plasma is transferred to a triple siliconized 20 ml volumetric flask which is then attached to an electric motor and rotated in a 37°C waterbath at 20 rpm for 10 minutes. For the investigation of plasma fractions 1.0 ml of the fraction to be tested is added to a suspension of washed platelets containing 5 x 10 platelets/µl in a saline-citrate solution containing 9 parts of isotonic saline and 1 part of 3.8 per cent trisodium citrate

After rotation 0.5 ml plasma are added to 3 ml of the saline-citrate solution. Two ml of this mixture are poured on each of two plastic slides and allowed to stand for 30 minutes – 0.5 ml of unrotated plasma or plasma fraction containing platelets are treated identically and serve as controls. After 30 minutes the slides are decanted and vigorously shaken in 200 ml of the saline-citrate solution to remove erythrocytes and leucocytes. For fixation the slides are immediately placed for 5 minutes in a staining cuvette containing 20 % formaldehyde Then the slides are carefully rinsed with tap water oxidized in 0 1 M potassium permanganate for 5 minutes rinsed again with tap water and stained with Giemsa staining solution (diluted 1 5) for 30-45 minutes. The slides are rinsed again in tap water and dried at room temperature Evaluation under the phase contrast microscope is done using 8-10 x magnification. In this test reversible platelet aggre gation means the sticking of the platelets to each other without fusion the cell membranes being intact Irreversible aggregation indicates that platelets are fused and membranes are partly destroyed.

Evaluation of the PAT

Both slides with rotated and nonrotated plasma are examined and graded according to the following characteristics

Grade 1 There are only single platelets, no aggregates.

Grade 2 After rotation, few reversible aggre gates are found there is no obvious re duction in single platelets (grades 1 and 2 are considered normal)

Grade 3 An increase in reversible aggregates is evident often with irreversible aggregation at the center the number of single platelets is

clearly diminished.

Grade 4 There are more irreversible than reversible aggregates single platelets are rare

Grade 5 There is complete or almost complete irreversible aggregation there are few reversible aggregates, single platelets are practically missing.

Normal washed platelets are aggregated in platelet-poor plasma (PPP) only of patients with enhanced aggregation (Grade 4 or 5) *Apparently a plasma factor is responsible for aggregation.*

We have tried to separate this spontaneous aggregating activity from the patient s plasma. This is possible using the following procedure to 1 ml of PPP 0,09 ml of 25 per cent ethanol (final concentration 2.1 per cent) are added at 0°C After *5 minutes centrifugation* at 0°C the sediment is resuspended in a citrate-saline solution. The platelet aggregating activity is found in the sediment which is rich in Factor VIII and contains about 5-10 per cent of the plasma fibrinogen The protein content of the sediment is higher if fractions are obtained from the *plasma of patients* with enhanced platelet aggregation. The supernatant plasma loses its aggregating activity (Table I)

Platelets resuspended in this supernatant can still be aggregated by ADP Spontaneous aggregation in patient s plasma and aggregation induced by ethanol fractions do not occur as rapidly as *ADP-induced* platelet clumping, but usually lead to complete irreversible aggregation of most of the platelets (Grade 4 or 5 of the PAT)

In normal PPP a platelet aggregating factor can be activated by glass contact or by addition of a number of mucopolysaccharides including heparin One of the most active substances is SP 54^R – a heparinoid at a concentration of 50 µg per ml PPP The ethanol fraction of SP 54-activated plasma aggregates platelets even when diluted five times. It contains more fibrinogen and more protein than the ethanol sediment of plasma which spontaneously aggregates thrombocytes.

If fibrinogen is removed from plasma by addition of thrombin and thrombin is subsequently neutralized by hirudin the plasma or its ethanol fraction lose their aggregating activity Hirudin alone has no enhancing or blocking effect on platelet aggregation.

Although these findings make it likely that the Platelet Aggregating Factor (PAF) is a fibrinogen derivative it has not yet been excluded that fibrinogen acts as a cofactor of another protein of high molecular weight. If plasma with PAT grade 4 or 5 or its ethanol sedimented fraction are separated by chromatography on Sephadex G 200 columns the platelet aggregating activity is found in the first peak, which contains fibrinogen but also lipoproteins Factor VIII and other macromolecules.

The ethanol sedimented fraction of a SP

Table I

PAT grades in platelet-poor plasma (PPP) and in supernatant and sediment after ethanol fractionation Protein and fibrinogen content in the sediment after ethanol fractionation (mean x and standard deviation s.d.).

PAT grade in PPP	in supernatant	in sediment	Protein in the sediment (mg %) x	s.d.	Fibrinogen x	s.d.	
2	2	3	93	15	24.7	1.1	3
3	2	4	132	49	31 7	11.0	38
4	3	4	172	77	36.0	17 7	38
5	3	5	255	201	29 6	12.3	8

54 – activated plasma still aggregates platelets after storage at +4 C or 20°C for 14 days. It can be lyophilized without loss of activity The ethanol fraction obtained from native plasma is less stable Its activity is usually lost after a few days. The model of mucopolysaccharide activated aggregation might serve to further separate and characterize the platelet aggregating factor(s)

Platelet Function in Afibrinogenaemia

Platelets from afibrinogenaemic patients do not spread on glass. They behave normally if a small amount of fibrinogen is added. If the platelets from afibrinogenaemic patients are washed and resuspended in Mg^{++} containing saline they also spread normally Washed normal platelets behave like the patient s platelets if they are resuspended in afibrinogenaemic plasma. They can not spread their adhesion on glass and ADP-induced aggregation are diminished. These platelets also regain their normal functions after renewed washing and resuspension in saline-citrate or in normal plasma or if fibrinogen is added. Washed normal platelets behave like afibrinogenaemic platelets if they are resuspended in defibrinated plasma or thrombin-free serum. Fibrinogen is apparently necessary for a "normal" platelet function in plasma. Platelet fibrinogen plays no decisive role If platelets are separated from the plasma they spread and adhere normally to foreign surfaces.

Gugler and Lüscher (5) thought that in afibrinogenaemic plasma possibly some proteins inhibit platelet function and that small amounts of fibrinogen overcome this inhibiting effect.

There probably exists a number of different plasma proteins which have either enhancing or inhibiting effects on platelet function or on platelet aggregation.

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3 PROTEIN REQUIREMENT FOR PLATELET AGGREGATION*

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Numerous studies (1 4 10,18) have dealt with the possibility of plasmatic cofactors being implicated in the aggregation process. More particularly fibrinogen has been thought to play an important part (5 12 14). In these studies either platelet-rich plasma (PRP) from patients with congenital afibrinogenaemia or extensively washed platelets have been used. With neither of these methods uniform results have been obtained.

We can make two contributions to this discussion

1 *Study of Platelet Aggregation in Two Unrelated Males with Congenital Afibrinogenaemia*

Two unrelated young males with congenital afibrinogenaemia have been studied. The results of various tests performed in both patients are shown in Tables I and II It can be noted that even with very highly sensitive methods, as that described by Merskey et al. (15) and that reported by Hawiger et al. (13) practically no fibrinogen was detected in platelet poor plasma from both patients. Tables I and II also show the values obtained after transfusion of 2 grams human fibrinogen (Belgian Red Cross).

Occurrence of clumping was followed with Born's photometric technique (3) In these two patients, aggregation by ADP (Sigma) thrombin (Roche) 0.3 NIH units/ml f.c., adrenaline (Stago) 10 μg/ml f.c. or Thrombofax (Ortho) 1/5 final dilution, a commercial substitute of platelet phospholipids capable of producing platelet aggregation (6) was never observed in basal conditions. All these substances were unable at the same concentrations, to aggregate normal platelets extensively washed in isotonic saline (see below)

Figure 1 shows the influence on aggregation by a relatively high final concentration of ADP ($5 \cdot 10^{-4}$ M) of the addition of purified human fibrinogen or fibrin monomer to PRP from one of the two patients. Purified human fibrinogen was kindly provided by Kabi fibrin monomers were prepared as described by Donnelly et al. (11)

In the absence of added human fibrinogen no aggregation was found (curve A) very small amounts of fibrinogen (0.04 mg/ml final concentration or more) added "in vitro" sufficed

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Table I

Plasma fibrinogen concentration (determined with 4 different methods) thrombin time and partial thromboplastin time in a patient with congenital afibrinogenemia. Effect of transfusion of 2 grams human fibrinogen.

	Normal range	Before fibrinogen	30 min. after transfusion
Plasma fibrinogen concentration (mg/ml)			
– Fibrin Polymerization Time Test (17)	2-4	< 0.08	0.54
– Blomback method (2)	2-4	< 0.50	0.55
– Tanned Red Cell Hemagglutination Inhibition Immunoassay (15)	2-4	0.0005	0.40
– Staphylococcal clumping test (13)	2-4	0.0001	0.32
Thrombin time (seconds)	18-24	> 240	22.5
Partial thromboplastin time (seconds)			
without activation with kaolin	<100	> 300	63
after activation with kaolin	<60	> 300	52

Table II

Plasma fibrinogen concentration (determined with 4 different methods) thrombin time and partial thromboplastin time in patient with congenital fibrinogenaemia. Effect of transfusion of 2 grams human fibrinogen

	Normal range	Before fibrinogen	30 min. after transfusion
Plasma fibrinogen concentration (mg/ml)			
Fibrin Polymerization Time Test (17)	2-4	< 0.08	0.45
Blomback method (2)	2-4	< 0.50	0.55
Tanned Red Cell Hemagglutination Inhibition Immunoassay (15)	2-4	0.008	0.64
Staphylococcal clumping test (13)	2-4	0.008	0.64
Thrombin time (seconds)	*18-24*	> *180*	*28.4*
Partial thromboplastin time (seconds)			
without activation with kaolin	<100	> 240	98
after activation with kaolin	<60	> 240	62

to restore aggregation by ADP (curves B and C) fibrin monomers (at a final concentration of 0.02 mg/ml) were devoid of spontaneous aggregating activity and were able to restore aggregability by ADP even better than fibrinogen (curve D)

Figure 2 indicates that transfusion of fibrinogen also resulted in corrected platelet aggregation by ADP. It has to be noted that before transfusion even very high final concentrations of ADP ($2 \cdot 10^{-4}$M) were not capable of producing considerable aggregation.

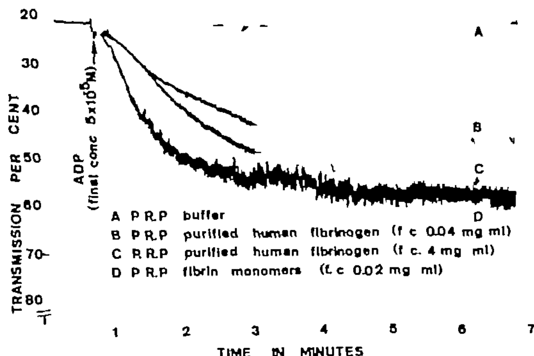


Figure 1

Superimposed tracings of aut matic recordings of platelet aggregation by ADP in patient with congenital afibrinogenaemia. Effect of the addition of purified human fibrinogen or fibrin monomers.

Figure 3 shows platelet aggregation elicited by a low final concentration (0.3 N.I.H. units/ml) of thrombin whereas before transfusion of fibrinogen no response was noted and PRP remained unclottable brisk aggregation was found after transfusion clotting was registered about 4 minutes after addition of thrombin (control values 40-60 seconds).

In contrast to the above mentioned aggregating substances, bovine fibrinogen (kindly provided by Kabi) aggregated afibrinogenaemic platelets in a normal way without any addition of ADP (Figure 4). It should be noted that at a final concentration of 0.8 mg/ml bovine fibrinogen provoked a double wave of aggregation as we have repeatedly observed in normal PRP (7) this suggests that under an appropriate stimulus, afibrinogenaemic platelets are capable of aggregating and of releasing ADP which would cause the secondary aggregation (7 16).

Our results would indicate that in our test situations fibrinogen is an essential cofactor for platelet aggregation. We cannot exclude however that in other experimental conditions as f.i. with concentrations of ADP higher than those used in this study (2.10^{-4}M) or with other preparations of the aggregating agents an appreciable aggregation of platelets from afibrinogenaemic patients would take place. Indeed, subsequent to this conference aggregation of the PRP of one of these afibrinogenaemic patients by the highly purified collagen preparation of Professors Hugues and Lapière (Liège) has been noted.

2. *Study of Aggregation in Suspensions of Washed Normal Human Platelets*

Suspensions of washed platelets were prepared by centrifuging PRP from normal individuals at 6,000 g for 10 minutes and gently resuspending

TRANSMISSION PER CENT

TIME IN MINUTES

A before transfusion final concentration of ADP 8×10^{-6} M

B before transfusion final concentration of ADP 2×10^{-4} M

C 30 minutes after transfusion final concentration of ADP 8×10^{-6} M

Figure 2

Superimposed tracings of automatic recordings of platelet aggregation by ADP in patient with congenital afibrinogenaemia. Effect of transfusion of human fibrinogen (2 grams). The arrow indicates the moment of the addition of ADP

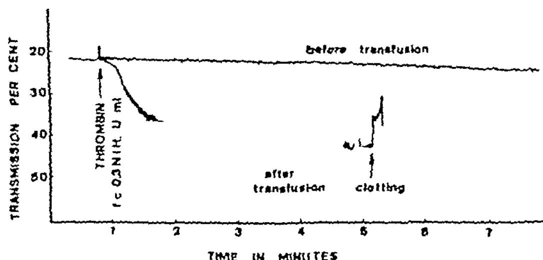


Figure 3

Superimposed tracings of automatic recordings of platelet aggregation by thrombin in a patient with congenital afibrinogenaemia. Effect of transfusion of human fibrinogen (2 grams).

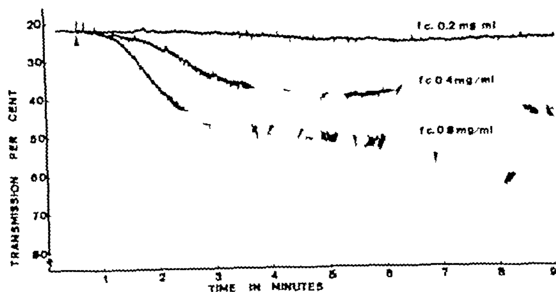


Figure 4

Superimposed tracings of automatic recordings of platelet aggregation induced by purified bovine fibrinogen (Kabi) in patient with congenital afibrinogenaemia. The arrow indicates the moment of the addition of bovine fibrinogen.

TRANSMISSION PER CENT

ADP or buffer

IgG

buffer and IgG

ADP and IgG

TIME IN MINUTES

Figure 5

Superimposed tracings of automatic recordings of the effect of ADP or buffer alone, Immunoglobulin G preceded by buffer and Immunoglobulin G preceded by ADP on clumping of a suspension of washed normal human platelets. ADP at final concentration of $5 \cdot 10^{-4}$ M Immunoglobulin G at final concentration of 0.015 % w/v

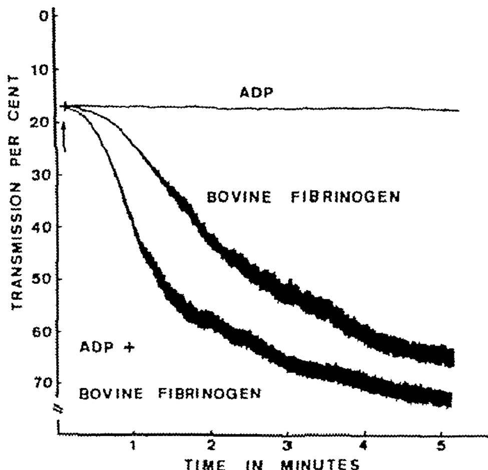


Figure 6

Superimposed tracings of automatic recordings of the effect of ADP or buffer alone bovine fibrinogen preceded by buffer and bovine fibrinogen preceded by ADP on clumping of a suspension of washed normal human platelets. ADP at a final concentration of $5 \cdot 10^{-6}$ M bovine fibrinogen at final concentration of 0.8 mg/ml.

the platelet button in a same volume of isotonic saline. This washing procedure was repeated three the final suspension was adjusted to 300 000 platelets/µl.

We have been studying an immunoglobulin G prepared from serum of a polytransfused patient with idiopathic thrombocytopenic purpura (8). This immunoglobulin preparation is capable of producing platelet aggregation by releasing platelet ADP. Whereas the washed platelets were not aggregated by ADP collagen or lysed washed platelets, they were readily aggregated by this immunoglobulin.

A strong platelet aggregation was also observed when purified bovine fibrinogen was added to washed platelets. Preincubation of washed platelets with various concentrations of purified human fibrinogen did not modify the aggregating activity *neither* of immunoglobulin nor of bovine fibrinogen.

As shown in Figures 5 and 6 both immunoglobulin and bovine fibrinogen make our preparation of washed platelets susceptible to aggregation by ADP. Indeed, earlier and stronger clumping was observed when the immunoglobulin or bovine fibrinogen was introduced subsequent to ADP than to buffer.

Clumping of washed human platelets by the patient s immunoglobulin and by bovine fibrinogen could be explained in one of two ways either the immunoglobulin or bovine fibrinogen by attachment to the platelet surface,

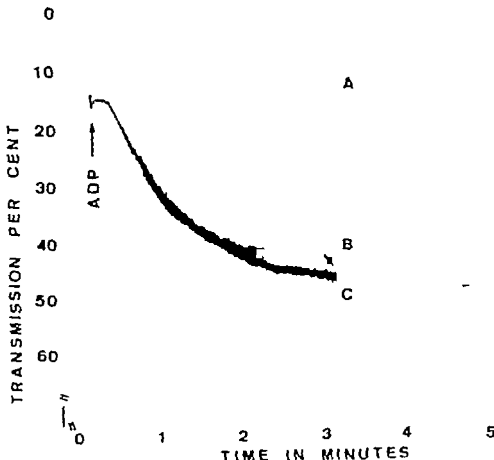


Figure 7

Superimposed tracings of automatic recordings of the effect of preincubation of afibrinogenaemic PRP with either human fibrinogen or bovine fibrinogen on aggregation by ADP (final concentration $5 \cdot 10^{-6}$ M)

A. effect of ADP human and bovine fibrinogen tested alone

B. effect of preincubation with human fibrinogen (final concentration 0.2 mg/ml)

C. effect of preincubation with bovine fibrinogen (final concentration 0.2 mg/ml)

produces the protein environment which would be necessary for aggregation by subsequently released ADP in this regard it may be emphasized that the immunoglublin, like bovine fibrinogen is capable of producing aggregation in afibrinogenaemic PRP and that concentrations of bovine fibrinogen unable themselves to provoke aggregation, render afibrinogenaemic platelets susceptible to aggregation by ADP (Figure 7) alternatively a reaction of immunological nature on the platelet membrane would produce such a modification of the latter that it regains aggregability by subsequently released ADP independently of the presence of protein cofactors. That this modification indeed occurs very rapidly is demonstrated by the pronounced increase of platelet factor 3 availability immediately after addition of the immunoglobulin (9).

In conclusion a cofactor of protein nature is required for aggregation of both afibrinogenaemic platelets and extensively washed normal platelets, at least in our experimental conditions. This cofactor however seems not to be strictly specific indeed we have observed that human or bovine fibrinogen and an -

immunoglobulin preparation reacting with platelets make both afibrinogenaemic and washed platelets susceptible to aggregation by ADP

Finally we would like to emphasize that very small concentrations of human fibrinogen are sufficient to restore aggregation by ADP in afibrinogenaemic subjects therefore it should be certain that the cases described as afibrinogenaemia are not in fact severe hypofibrinogenaemia, in which platelet aggregation should be relatively normal One of our patients on one occasion had a spontaneous slight rise of his plasma fibrinogen level (to 0.08 mg/ml) At that moment, platelet aggregation by different aggregating agents was normal.

Acknowledgements

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DISCUSSION

J HUGUES I should like to ask Dr Born a question about his in vivo experiments. Is there no contact between platelet and basement membrane ?

G V.R. BORN No there is no contact that we can see on electron micrographs. The cell lining is completely intact we have taken pictures right round the vessels and there is no gap

J HUGUES You have injected ADP intra venously ?

G V R. BORN No on the outside This, of course raises many questions Is it the ADP that initiates the whole process and if so how does the ADP get through the wall ? Or does ADP change the endothelial cell so that platelets then adhere to it ? But there is no gap and no damage. Control experiments show that, under the same conditions, guanidine-diphosphate or phosphoric acid have no effect

J HUGUES I should like to show some pictures concerning the very early stages of ADP action on platelets.

The platelets are fixed for electron microscopy 8 to 10 seconds after the addition of ADP to PRP before any trace of aggregation is visible At this point, some threads bridging the free platelets (Figure 1) are seen These structures are shortlived and disappear when platelet clumping starts. We don t know the nature of these threads. We have never observed such structures in normal PRP or PRP exposed to thrombin or collagen If PRP is previously incubated with prostaglandin E_1 before ADP is added the bridges do not appear (Figure 2) They are also missing with PRP from an afibrinogenaemic patient exposed to ADP (Figure 3)

E.F LUSCHER Dr Hugues, do these threads which seem to develop very rapidly persist during aggregation ? With the electron microscope Parmeggiani was never able to see any thing like that in the early phases of aggregation

J HUGUES These threads were visible just in the first few seconds after contact with ADP and before aggregation occurs. They disappear during the aggregation process.

S BYGDEMAN Professor Born pointed out that the platelet release reaction is much too slow to play an important role in the immediate platelet-endothelial interaction. In a series of experiments on nembutal anesthetized rabbits we have injected 200 and 600 mg/kg of acetylsalicylic acid dissolved in THAM-buffered saline and determined the effect on ADP and collagen-induced platelet aggregation measured with the turbidometric method. As we expected a marked decrease in collagen-induced platelet aggregation was obtained while the effect of ADP was not significantly re duced In a second series of nonanesthetized rabbits the immediate platelet-endothelial inter action was studied in vivo at the site of a bio-laser induced endothelial trauma according to the method described by Arfors et al. (1)



Figure 1

Platelets from sample of normal PRP fixed for electron microscopy 8-10 seconds after addition of ADP some threads bridging the free platelets are visible Final magnification 5,800 X. (Electron micrograph taken by Dr L.J Simar Institut d Anatomo-Pathologie (Prof E.H Betz) University of Liège).



Figure 2

Platelet from sample of normal PRP previously incubated with PGE_1 and fixed for lectron micro-scopy 8-10 seconds aft addition of ADP the bridges visible in figure 1 are no more present Final magnification 4 900 X (*Electron micrograph taken by Dr L J Simar Institut d'Anatomo-Pathologie (Prof. E H Betz) University f Liege*)



Figure 3

Platelet from patie ith ongenital fibrinogenaemia, fixed f lectron microscopy 8-10 seconds fter addition f ADP to PRP no bridges bet een the platelet ar isible Final magnification 17,500 X (Electron micrograph taken by Dr L J Simar I titut d Anatomo-Pathologie (Prof E H Betz) University f Liege)

these experiments injection of 200 mg/kg of acetylsalicylic acid did not affect platelet aggregation at the site of endothelial trauma I think these results support the view of Professor Born that the release reaction in the classical sense does not take part in the immediate platelet endothelial reaction although the results do not exclude the possibility of an important role of the release reaction later in the process of thrombus formation.

G V R. BORN This is very interesting. Could I make quite sure that by release reaction I mean the release of material from the specific storage granules. You may get ADP released from the membrane but I do not call that a release reaction. I think we are all clear about the distinction. The release reaction is what you see as a second hump in the optical density curves.

J.R. O BRIEN Professor Born may I raise a point about the rapid shape change you have mentioned. In the exciting photomicrograph of the inside of a blood vessel you showed there were about 10 platelets which seemed not to have changed their shape How do you explain this, since ADP presumably was the trigger mechanism ?

G V R. BORN Well, that is also what we think. But I do not want to be dogmatic about this because this will have to be quantitated and we are going to do this. At present, my impression is that platelets can adhere *in vivo* without the shape change

J.R. O BRIEN Were these platelets stuck ?

G V R. BORN Yes, I think so otherwise we would not catch them on the electron micrographs

J.R. O BRIEN They did not seem to be very close

G V.R. BORN They look very much like an initial aggregate You would not see them like that if they were not stuck there are quite a number close to each other With such considerable gaps apparently between them, the question is whether they are in contact The only way to get at this is by careful serial sectioning which is what we are doing

E.F LUSCHER I would assume anyhow that before morphological alterations become discernible by electronmicroscopy the membrane must undergo alterations leading to a rearrangement on a molecular level and involving an altered charge distribution I think it is not prerequisite that you must see something in order to prove a structural alteration of the membrane

G V.R. BORN I agree with that We are interested in this because there is much discussion whether or not adhesion of any kind of cell requires it to show out pseudopods. A hypothesis by Bangham is that various cells adhere to each other when they touch by means of long microspikes or microvilli Platelets can produce such microspikes and the crucial question is whether platelets must produce them before they can adhere to the vessel wall or to each other

R. GROSS Dr Born have you any observations or ideas whether these early changes in the shape depend on the energy metabolism of the platelets, because we know from the general biology that any other form than the spherical one is an energy consuming situation

G V R. BORN What I suggest is that just like the normal erythrocyte the shape of the normal platelet is a thermodynamically unlikely state Of course this normal shape may also depend on ATP being present stoichiometrically without turn-over in the membrane this is perfectly possible The more probable state comes about when platelets have undergone the shape change That is the reason why almost anything done to them will alter their shape if you wash them or cool them, or add EDTA or metabolic inhibitors, the slightest kick will turn them from this normal thermodynamically

improbable shape to the thermodynamically more probable shape Unfortunately with a whole cell system it is not possible to analyse this in terms of activation energies and entropy changes.

A.A. SHARP This debate on shape change and aggregation is one that we have had for many years and in relation to work done by John French in Oxford we came to the conclusion that when the platelets aggregate they return to their original shape I think you can see under phase contrast as well as electron microscopy that when they clump the platelets lose their spidery appearance and become disc-shaped again very quickly and suddenly This point relates to the picture you showed of in vivo aggregation on the vessel wall and your comment that the platelets do not show a shape change I think there is a distinct possibility they did undergo a shape change and then having aggregated assumed their old disc-like shape.

G V R. BORN It may be so but then they have to do it like lightning This particular picture was obtained like this the current was switched on and as soon as the hand was taken off the current button gluteraldehyde was dribbled on We reckon that the effect happend in about 10 seconds, that is very quickly

A SHARP I think this is entirely possible

E F LUSCHER Ten years ago Parmeggiani looked at the early phases of thrombin induced aggregation with the electron microscope The reason was that we hoped to find evidence for the extrusion of contractile protein from aggregating platelets. This would beautifully explain clot retraction and the spontaneous contraction of loose aggregates as seen by Sokal by means of phase contrast microscopy We never saw anything like that the platelet membrane although there were gross morphological alterations, remained intact and smooth This is in contrast to Dr Hugues pictures which clearly show a fibrous material between the platelets. I wonder whether this is also observed with washed platelets in what suspension medium were these pictures obtained ?

J HUGUES They were taken in PRP with platelets exposed to ADP and very early after the exposure It may be the protein required for aggregation which precipitates between the platelets.

E.F LUSCHER You are of course aware of the fact that many people including Dr Born have postulated bridges formed from fibrinogen together with ADP I might add that we have looked at this and found that ADP certainly does not form any complexes with fibrinogen

G V R. BORN Again one has to distinguish carefully between the physiological and the artificial As far as I know the evidence at present is that a number of different substances including proteins can bring about aggregation artificially The question is, what are the physiological cofactors ? The evidence suggests that fibrinogen is one of them. We like others, have evidence that there is at least one other cofactor Dr Bang has evidence for Hageman factor factor V and gammaglobulins

J HUGUES Thrice washed platelets exposed to collagen fibers adhere very well to these fibers but the platelets neither spread along the fibers nore fuse together If a small amount of adsorbed plasma is added to the platelet suspension, fusion and homogenization of platelet mass normally occur clearly demonstrating that a plasma factor must be present

J CAEN I have just one comment on this spreading of platelets on collagen fibres and on glass I think that in congenital afibrinogenaemia we can find spreading on collagen fibres and not on glass and if you add a minor amount of fibrinogen, it is quite enough to have the spreading I believe that at least the plasma fibrinogen is important in this condition I just want to come back to the experiment of Born and ask him if he was able

to measure the ADP in the vessel indeed, I remember the work done in other conditions by Speat some years ago and it seems that, in the experiment by Spaet, ADP was inside the vessel and the rate of disappearance of ADP in the vessel was very important. If ADP is in the vessel I think that it could be an argument against the fact that the vessel plays a role in this kind of aggregation Were you able to measure ADP after the infusion ?

G V.R. BORN No we were not, because we are trying to get to the limit of what can be done That is one of the reasons why we are using this technique The amounts that come out of those micropipettes are extremely small a very interesting quertion is, as I said before whether the ADP itself causes what we see and, if so whether it goes through or between the endothelial cells. One would think from general evidence that it should not go through but between the cells. We have looked very carefully at the gaps at the junctions and have seen no abnormality There are ways, we hope of measuring how much ADP gets into the vessel. But then comes the very interesting point if ADP gets into the vessel, where is it and how much is there other than a few molecules next to the wall The blood flow in these vessels is very fast and in fact we have got some results on the relation of blood flow to the rate constant of this growth. The fact is that the blood flow is so fast that any ADP that is further than the hypothetical stationary layer of plasma away from the wall of the vessel would be diluted and just washed away So the question really is how much ADP is right next to the vessel wall. I would guess that the amounts are quite ridiculously small. We hope to be able to give a quantitative answer later

J CAEN With which animal was this experiment done ?

G V R. BORN It was with hamster cheek pouch.

J CAEN Depending on the type of animal used you have quite different modifications of ADP in the blood for instance as you know if you use a rat, the disappearance of ADP is much more rapid than in a hamster or a guinea pig possibly even if you use minute amounts of ADP the rate of the disappearance of ADP is very low

G V.R. BORN Yes certainly but the enzyme effects are likely to be very slow compared to the effect of the blood flow which is fast and presumably washes the agent away The amounts are likely to be very small in the vessel but that still needs investigating.

E.F LUSCHER Is ADP not a vaso-active substance ?

G V R. BORN In the hamster cheek pouch ADP constricts the vessels, particularly the small arteries, whereas ATP and AMP are both vasodilators we do not know why

E.F LUSCHER This implies that ADP must combine with some receptor most likely on the endothelial cell. This might alter the cell in such a way that it becomes attractive for the platelet. What do you think about that ?

G V.R. BORN Any caliber change would be due to an effect on the smooth muscle in the wall, not on the endothelial cell. So I do not think you can argue from changes in the caliber of the vessel to effects on the endothelial cell. On the other hand, as I was careful to point out, there is a change in the endothelial cell which we are trying to investigate

E.F LUSCHER I want to point out that the endothelial cell itself is a smooth muscle there is no doubt about that. Majno and others have shown this quite convincingly I would like to comment on Dr Hugues last two slides In order to have fusion of the platelets and formation of denser aggregates a plasma cofactor most likely fibrinogen, is required. Collagen itself can only affect those platelets which adhere directly to it. Whatever happens later on

depends again on reversible followed by second phase aggregation there the plasma cofactor is required, otherwise nothing is going to happen. Would you agree with that ?

J HUGUES Yes, I agree

E.F LUSCHER I have some comments on Dr Breddin s presentation it is well known that complexes formed from fibrinogen degradation products and unchanged fibrinogen molecules are more powerful aggregating agents than pure fibrinogen and I would also point out the work by Solum who has shown that in the course of fibrin polymerization extremely active products are formed. Platelet factor 4 in combination with such fibrinogen complexes seems particularly active The formation of such complexes, even under physiological conditions is possible but nobody knows at present whether this is of functional significance I think there is no reason for being too much amazed of the fact that there exist derivatives of fibrinogen which are more active than fibrinogen itself

G V R. BORN Professor Roka has shown that procollagen, the soluble precursor of collagen, is a potent aggregating agent for platelets and he has suggested that small concentrations of procollagen in the vessel wall may be much more important than collagen in their effect on platelets.

R.M HARDISTY To return to the question of fibrinogen Hutton and I (2) studied platelet aggregation in a patient with afibrinogenaemia, and found practically no aggregation with ADP at a final concentration of μM When fibrinogen was added to a final concentration of 40 mg per 100 ml aggregation occurred normally with this concentration of ADP and as little as 11 mg per 100 ml allowed some aggregation At higher concentrations of ADP however (20 and 200 μM) quite good aggregation occurred in the complete absence of added fibrinogen These findings therefore do not seem to suggest that there is an absolute requirement of fibrinogen for ADP aggregation but only that it is necessary for aggregation by relatively low concentrations of ADP

R. GROSS In our case of afibrinogenaemia we had a nearly normal aggregation and the problem is to my knowledge that all people who describe cases of afibrinogenaemia find traces of fibrinogen by immunological methods. My question is whether traces of fibrinogen detected by immunological methods have been excluded it may be that this small amount of fibrinogen is efficient on the platelet surface

R.M HARDISTY We found no fibrinogen whatsoever by Tanned Red Cell Hemagglutination Inhibition Immunoassay

E.F LUSCHER Dr Hardisty did your patient have a pronounced bleeding tendency this generally goes along with exceedingly low fibrinogen levels.

R.M. HARDISTY Yes certainly she has.

M.J LARRIEU May I come back to Dr Breddin s work and Dr Luscher's comment about fibrinogen and fibrin derivatives. We must clearly distinguish between the role of fibrinogen as a cofactor of platelet aggregation and the effect of some fibrinogen derivatives on platelets. We have studied *in vitro* the fibrinogen derivatives found by Dr Breddin in patients with thrombosis and which were also described by the Polish Group as soluble complexes" These derivatives or complexes (as we reported in Leuven two months ago) do not act as a cofactor of platelet aggregation but are able to induce a platelet release reaction as well as collagen or antigen-antibody complexes.

G V R. BORN That raises the exact point I made at the beginning. I think we must try to distinguish as carefully as we can between any kind of artificial system and the physiological system. At the moment it is very difficult for us to make this distinction because we all work with various artificial systems. You can get release with all sorts of things and all sorts of

proteins may have an effect. The question is how can we work our way from there to the walls of the forbidden city i e the physiological situation. Clinical situations are of course physiological experiments. What happens for example in multiple myeloma ? When gammaglobulins are in vast excess do they have any effect ?

H REUTER I wanted to make a remark to the observation of Dr Hardisty The phenomena you have observed may depend on a simple reaction of fibrinogen with ADP resulting in an activated form of fibrinogen. If the concentration of one of the reactants, in this case of ADP is increased the reaction may be shifted to the side of the activated form, in this case an aggregation could take place

E.F LUSCHER This is quite an interesting idea. Do you have any evidence for this ? Unfortunately equilibrium dialysis, at least in our hands, lends no support whatsoever for a binding of ADP to fibrinogen The possibility remains that ADP induces in the fibrinogen molecules a subtle change leading to an activated form. Do you think something like this might happen ?

H. REUTER I think so but I have no experimental evidence for it.

R.M HARDISTY In reply to Dr Reuter in our case we simply found no fibrinogen at all by any method, so that one could have to postulate the formation of an activated form of something that was not there or which was not detectable by the methods that we used. One thing which I should add is that in this patient we found perfectly normal aggregation with collagen.

G V.R. BORN What is the minimum fibrinogen per 100 ml of platelet-rich plasma measurable by the most sensitive methods

R.M HARDISTY The answer to that is certainly less than 1 mg per 100 ml which is very much less than the smallest amount required for ADP aggregation

G V.R. BORN Dr Hardisty I would like to ask if you believe in some sort of protein cofactor at all If it is not fibrinogen, could it be that some fibrinogen derivative which you do not pick up by immunological methods, a precursor or variant or some other protein, can take the place of fibrinogen in aggregation ? Is it possible that immunological methods do not pick up something which is active but has different antigenic determinants ?

R.M HARDISTY I think this is possible One thing we have not done on this patient is to look for the presence of an abnormal fibrinogen. These results were obtained several years ago and I have not had the opportunity of doing this.

G V.R. BORN But it is a possibility ?

R.M. HARDISTY Yes.

J VERMYLEN We have been able to study a patient with congenitally abnormal fibrinogen and another patient with hepatoma and acquired abnormal fibrinogen molecule In both instances, fibrinogen could readily be detected in plasma with the immunological method Dr Hardisty has been using (the tanned red cell hemagglutination inhibition immuno-assay)

J CAEN With regard to Professor Gross question we have had the opportunity to see four cases of so-called congenital afibrinogenaemia. A very severe one had less than 1 mg per 100 ml at least when immunologically examined by Seligman In this patient, I confirm completely what Dr Hardisty has shown. Depending on the dose of ADP you use you can have an abnormal aggregation, but if you take 100 times the usual range of ADP you obtain a normal aggregation. The same is true with thrombin also and in this regard we have not the same results as Dr Vermylen

In our case using 0,33 g/l (final concentra-

tion) of bovine fibrinogen from Kabi or Boehringer we obtained recently a normal aggregation.

Now coming back to Dr Breddin s problem, when he says that there are some modifications after the washing of platelets, this is quite true as after washing platelets, even thrombasthenic platelets, you can have a spreading on glass. So I think that after washing there are modifi cations occurring on or in the platelets and I agree with Professor Born when he mentioned that it is sometimes very difficult to compare what occurs in platelet-rich plasma and in washed platelets.

R. GROSS I would only give some remarks to Dr Born s question. It may be that in afibrinogaemia some products play a part in the release reaction but are not detectable with other methods of fibrinogen examination.

H HOLMSEN In most of the experiments presented here on aggregation of afibrinogenaemic platelets, aggregation occurs nearly only during conditions under which the release reaction takes place My question is could it happen that fibrinogen was released and ac tually acted as the necessary plasma cofactor ?

A.S DOUGLAS I would like to make one brief comment Professor Born mentioned the possibility of studying ADP reactivity of plate lets in patients with myeloma and that this might shed some light on related plasma factors additional to fibrinogen I can report on one patient with macroglobulinaemia in whom there was marked impairment of ADP reactivity of the platelets these were studies carried out by my colleagues in the University Department of Medicine Royal Informary Glasgow

K. BREDDIN We thought that platelet fibrinogen might play a role in this we took normal platelets, washed them once and put them into the plasma of a patient with afibrinogenaemia and then they behaved like this patient s platelets. You can do this several times, you can take the platelets, wash them once then they will spread, put them back into the patient s plasma then they will lose their spreading capacity again You may wash them again and they will again spread. I think it is very unlikely that just changes in the platelets are responsible for these quite consistant results, and the normal platelets which have of course the normal platelet fibrinogen behave in the patient s plasma just like the patient s platelets as far as spreading and aggregation to ADP is concerned.

E.F LUSCHER I think this part of the discussion has come more or less to an end. Let me summarize quite briefly in my view ample evidence has been presented that fibrinogen is a cofactor of platelet aggregation by ADP and that perhaps aggregated fibrinogens are more potent factors. It is noteworthy though that as pointed out by Dr Larrieu, certain fibrinogen complexes may aggregate platelets directly without the primary requirement of ADP With respect to other plasma cofactors we have heard about Bang s finding about factor XII and perhaps still other plasma proteins.

I am wondering how this fits in with the observation that in severe afibrinogenaemia, i.e in people with an adequate supply of factor XII aggregation just simply does not take place I my view this proves that fibrinogen still is the required plasma cofactor for ADP We certainly should pay attention to other products wich may be derived from fibrinogen and which might show properties other than those of a cofactor for ADP and which might be of considerable importance for the understanding of platelet aggregation

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Question N 2

WHAT DOES THE OPTICAL PLATELET AGGREGATION TEST ACTUALLY MEASURE ?

DISCUSSION

J R. O BRIEN
G V R BORN
P.M. MANNUCCI
A. SHARP
J CAEN
R. GROSS
J.J SIXMA
H. REUTER
R.M HARDISTY
J HUGUES
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4 WHAT DOES THE OPTICAL PLATELET AGGREGATION TEST ACTUALLY MEASURE ? INTRODUCTORY REMARKS

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The optical platelet aggregation test is undoubtedly a very easy and practical method. But it must be stressed that this photometric technique used by many workers does not exactly reflect the aggregating action of the clumping agent as already pointed out by many workers.

The curve obtained (Figure 1 total OD) represents the sum of two simultaneous phenomena the fall in the number of free platelets and the increase in the number and size of the clumps A free platelet count can be performed on samples from the reaction tube at any moment of the aggregating process (Figure 1 Pl. Nbr) and the corresponding curve of their optical density determined (Figure 1 Pl. O.D) on the basis of previous experiments with various dilutions of the same PRP Then the real curve (Figure 1 clumps O D.) corresponding to clump formation and subsequent dispersion can be drawn by substraction from the total O D curve This curve is quite different from the experimental photometric curve

The turbidimetric technique is also misleading. Under other experimental conditions, for example the optical density decreases without concomitant clumping when the free platelets are completely emptied of their contents by exposure to certain substances.

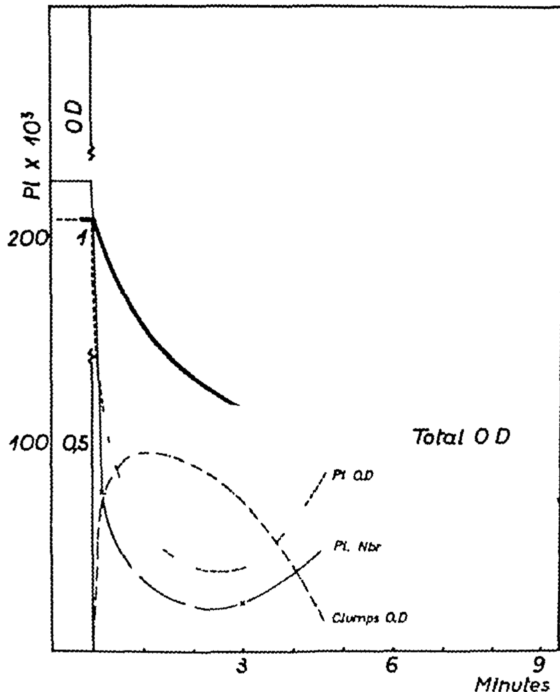


Figure 1

Optical density (O.D.) ariation in PRP (3.3 ml) + ADP (0.3 ml final oncentration 2 10^{-4} M)

x x Free platelets count

O.D variation orresponding t free platelets

-- Theoretical O.D variation orresponding t the ggregates

5 MODIFICATION OF SHAPE AND VOLUME OF PLATELETS IN THE EVALUATION OF PLATELET AGGREGATION TEST

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Some people have made mathematical models of what happens but perhaps can I just draw your attention briefly to work that Michael Hume and I did in 1967 (2) The idea at that time was the optical density curves were useful but entirely empirical things no one knew what they meant in terms of aggregates We aggregated platelets in the aggregometer fixed them by putting into the stirred plasma formalin to a final concentration of 1 or 2 per cent, and we showed that this stopped the aggregation curve within a second or two and that the aggregates were then fixed. Then we took out samples, put them into hemocytometer chambers, photographed them and made photomontages. On these we got large numbers of cells and aggregates so that the results were statistically valid. We counted pairs, triplets, quadruplets, and so on, quite easily all the way up to aggregates of eight platelets with more than eight we gave up Single platelets together with those in aggregates upto eight we called countable platelets. Beyond that we measured sizes of the aggregates by drawing around their outlines this provided a distribution of aggregate sizes. By knowing the total number of platelets and the number of countable platelets we obtained the total number of uncountable platelets present in the larger aggregates. The essence of the result was that when the optical density had decreased only very little Le less than 5 per cent single platelets and small aggregates had already almost disappeared In the second phase the dense packing of the aggregates increased greatly this is well known from electron microscopy We were able to calculate that the dense packing was almost 90-95 per cent, the maximum possible

Therefore very small changes in light transmission accompany considerable aggregation This has been brought up against Haslam s evidence that thrombin aggregation is inhibited by systems that remove ADP (4) because apparently inhibited curves do show a slight decrease in optical density

I now come to the rapid change reaction as recorded by the optical density method The shape of the curve by ADP is characteristic It goes through a maximum, then falls to a plateau and then there is a further slow decrease in light transmission When plasma is diluted with distilled water to such an extent that the platelets swell but do not lyse the rate of osmotic hypotonic swelling can be compared with the rate of the shape change as recorded optically The reason for doing this is that, according to several people (3,5,6) the rapid shape change is associated with an increase in

platelet volume The evidence is entirely based on the Coulter Counter that is, the shape change is associated with a shift to the right in the coulter-counter curve I now have evidence that this does not represent an increase in mean volume (1) Mean platelet volume was measured directly in little thrombocrit tubes. Each tube consists of a reservoir which holds 0 6 ml of platelet-rich plasma. The tube is of a size that fits the high speed head of the MSE centrifuge so that it can be centrifuged at high speeds. The reservoir is attached to a capillary by smooth shoulders so that no platelets settle there The capillary is 0.9 mm in diameter and the glass is thick that the capillary is seen enlarged as in a clinical thermometer If platelet-rich plasma is centrifuged in these tubes at 10,000 g before and after the shape change is induced by ADP the results are as follows. Any red cells spin into the bottom of the tube and separate almost completely from the platelets which form a white column After ADP the column is higher At first I thought that that confirmed conclusion of others that there is an increase in mean platelet volume But when either radioactively labelled inulin or radioactively labelled albumin was added to the plasma before centrifugation the results showed conclusively that the increase in height of the column (which can be measured to 0 5 mm) is entirely due to an increase in extra-cellular plasma volume and not to any increase in the volume of the platelets themselves. So it can be concluded that the optical record is due entirely to the shape change and not to an increase in volume and that the interpretation of the Coulter-Counter results is wrong. After the platelets have changed shape presumably they cannot pack so closely together any more and therefore the extra-cellular volume is bigger (by up to 60 per cent). As control, we demonstrated that when platelets swell in hypotonic solution, an increase in mean platelet volume is indeed measurable

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6 FACTORS INFLUENCING THE OPTICAL PLATELET AGGREGATION TEST

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The most general question is what does this optical method measure First of all I think it is most important to realize that one is working with platelet-rich plasma (PRP) and that this raises the long and complicated argument about which platelets are you studying. We compared platelet counts made on whole blood and on platelet-rich plasma to see how many platelets we had lost in the preparation of the platelet rich plasma. In quite a high proportion of these observations no platelets were lost at all, but on the other hand sometimes up to 30 per cent of the platelets had been lost. This is a very important practical point whether the plate lets that are lost are special platelets why in the majority of cases you do not lose any and *why sometimes you lose a lot. I do not fully* know the answer to any of these questions exept that I did study carefully a whole lot of platelet function tests comparing the samples in which I had lost no platelets with the samples in which I had lost a lot of platelets and I could find no difference I found no evidence that I had lost particularly sticky platelets.

My observations on the importance of single tons were made long ago (1) using roughly the same technique as Professor Born. I then concluded that the increase in optical density as ADP induced aggregation progresses runs re markably parallel to the decrease in singletons, with polymers and large aggregates playing a relatively small part.

A very recent observation by Mr Etherington, my collaborator may help those who try to quantitate platelet response Many workers have chosen to adjust the platelet count to some arbitrary constant figure by diluting with platelet-poor plasma before adding the aggre gating agent This takes time and could also introduce errors due to handling the plasma We (3) chose to aggregate the PRP as obtained by centrifgation as quickly as possible and to measure the "slope" (raw data slope) We then read off a graph what the slope would have been had the count been 400,000 per c.mm. We called this the corrected or adjusted slope The *graph lines were obtained previously by diluting* many samples of different PRP's and measuring the slopes of all the dilutions with different counts.

It now seems that the raw data slope divided by the number of platelets in the PRP is closely related to the adjusted slope We now think this derived figure is probably a more accurate way of measuring the aggregability per platelet which in any case should be proportional to the aggregability per 400,000 platelets. So we may consider (1) the overall aggregability of a sample of PRP – the raw data slope and (3) the aggregabil ty per platelet and I do no

which if either of these two figures is more relevant to physiology

It might be useful to have a list, probably incomplete of the factors influencing platelet aggregation Obviously the number of platelets determines the number of successful collisions the speed of stirring also has considerable effect then the shape and the size of the stirrer and of the container will I am sure all make a difference to the type of tracing obtained. If citrated blood is used obviously the calcium concentration or the degree of citration will have an effect. Other factors are the temperature the pH, concentration of the aggregating agent and finally what I suppose we are all interested in namely the aggregability or the reactiveness of the platelets themselves.

I would mention another situation (2) which may help us to analyse fully the contribution of singletons, dimers etc. If alcian blue final concentration 0.5 mg/ml is added to a suspension of 100,000 washed human red cells per c.mm. in saline at 37°C and the suspension is stirred in my platelet aggregometer then red cell aggregation occurs giving a straight line tracing similar to that when platelets are aggregated by ADP at 15°C thus this situation is not complicated by disaggregation.

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7 LIGHT SCATTERING AND PLATELET AGGREGATION

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The nephelometric technique has been in use for the measurement of platelet aggregation since 1962 (1,2). The analysis of the optical records revealed that in addition to the greater transmission of light through the platelet suspension a small initial decrease in light transmission is often present just prior to aggregation It has been suggested that this initial decrease in transmission is caused by the change in platelet shape from discoid to spherical (4,5)

Professor Born has published a comprehensive study dealing with this initial rapid reaction of platelets brought about by ADP and preceding the aggregation Briefly the method described the use of suitable platelet dilution and the presence of EDTA to prevent platelet aggregation. Born also clearly demonstrated that the shape change is not accompanied by an increase in platelet volume (3)

Recently Born and I have been investigating a modified photometric technique which we believe will give us further information about the platelet behaviour just prior to their aggregation. The technique allows the observation of the initial shape change and the subsequent platelet aggregation to be carried out in the same sample of platelet-rich plasma (PRP) by the simultaneous measurement of light transmitted and scattered by the suspension of platelets. It occurred to us that a suspension of particles such as platelets in PRP would scatter light and that any change in shape of such particles should be accompanied by a corresponding change in the light scattering property of such suspension

We have incorporated a second photocell into an aggregometer to allow the measurement of the amount of light scattered by the platelet suspension The results presented here describe the measurement of light scattering at right angle to the incident beam. The amount of light transmitted through the suspension together with the amount of light scattered is recorded on a twin channel potentiometric chart recorder

Figure 1a represents a typical response of human platelets in citrated platelet rich plasma to the addition of ADP (0 5 ml PRP diluted with 0 5 ml 0 154 M saline) The top trace was recorded by the photocell in the transmitted light path and shows the small initial decrease in light transmission which preceded aggregation and the increase in transmission associated with platelet clumping. The lower trace represents the light scattered at right angles to the incident beam. This record shows a rapid decrease in scattered light followed by a lag phase or plateau and, finally a further decrease in the amount of light scattered. The second decrease in scattered light corresponds to the increase in transmitted light associated with aggregation. When EDTA is present in order to

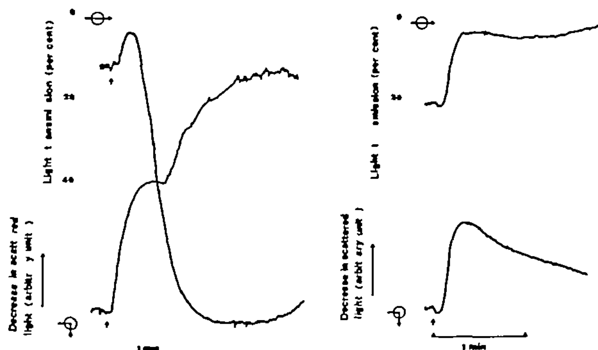


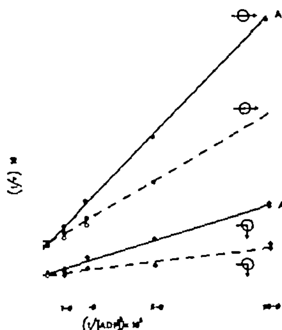
Figure 1

The effect of ADP on the transmission and scattering of light through plasma containing platelets.
Human PRP was diluted with saline (0.5 ml PRP and 0.5 ml 0.154 M saline). Final platelet concentration was 1.56×10^8 platelets/ml. Temperature = 37°C.
a) Changes in light transmission (upper trace) and changes in light scattering (lower trace). ADP final concentration was 1 μM.
b) Similar experiment but in the presence of EDTA (final concentration of 2 mM).

prevent aggregation. ADP causes a rapid decrease in both channels and the rapid decrease in transmitted light follows a similar path to the decrease in scattered light (Figure 1b).

We measured the velocity of the first phase of platelet aggregation and the initial velocity of the decrease in scattered light after the addition of various aggregating agents. Substances such as 5-hydroxytryptamine, thrombin and collagen also cause the initial rapid shape change. In Figure the changes caused by ADP in plasma containing platelets are expressed as double reciprocal plots of 1/velocity and 1/ADP concentration. In the presence of adenosine the velocity of aggregation and shape change is decreased. The results indicate a competitive inhibition by adenosine of platelet aggregation caused by ADP. Similarly the initial velocity of the change in light scattering induced by ADP alone and ADP in the presence of adenosine show a remarkable tendency for a common intercept on V_{max} axis. This in itself may not prove competitive inhibition at a common receptor-site but it certainly suggests some degree of competition between ADP and adenosine on both aggregation and the shape change.

The experiments described here indicate that the initial change in shape which platelets undergo when exposed to aggregating agents, can be studied by measuring the scattering of light by the cell suspension. The measurement of transmitted light simultaneously allows us to follow the course of platelet aggregation and their initial shape change independently of each other. These observations give us additional information about the behaviour of platelets in vitro and can be used to assess quantitatively



F gure 2

Double reciprocal plots for velocity of shape change (lower lines) and velocity of platelet aggregation (upper lines). Presence of adenosine is indicated by A and solid lines. Adenosine (1 µM final concentration) was added 2 minutes before ADP.

the effect of various substances on platelet function.

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8 A MATHEMATICAL MODEL FOR OPTICAL PLATELET AGGREGATION TEST

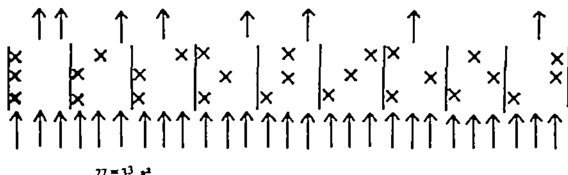
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When aggregation occurs in platelet rich plasma the platelet mass is always the same therefore the reason why more light is transmitted is due to a different distribution of the platelet mass in the course of aggregation, so that light beams might pass through *between* the platelets (plate let aggregates). In figure 1 I have considered a number of particles that could be placed in one layer but instead was equally distributed in three levels. In this condition some particles come behind one another so that light will be transmitted between these particles. In this example where we place the platelets of one layer in three levels, out of 27 beams, 8 beams come through. Certainly most of the light might be scattered and not absorbed, but it is the transmitted light I am interested in. One can make it much more complicated and add several layers arranged in several levels, etc. and Table I shows the general formula. Here a represents the possibility of positions in different levels. That means that in an ordinary test tube it is a very large number The equation will therefore go towards a limit value for every value of layers" This is dependent on the number of particles and the expression can therefore be simplified to the logarithmic expression of the Table I

If we also consider how aggregation might occur we can see that at first, when there are many platelets and many small aggregates, there will be an intense number of collisions, but, as time goes on the number of individual particles will decrease and therefore the rate of collisions will diminish and aggregation will get slower The number of aggregates will therefore decrease inversely proportional to time (Table II). If these two expressions are combined you will get the formula of Table III This takes into consideration the light transmission through a particle suspension and the assumption that the rate of aggregation is proportional to the rate of collisions. This expression will yield a straight line on a semilogarithmic paper (Figure 2). You can also put it on an ordinary paper and you will see that the resulting curve resembles the primary wave of aggregation (Figure 3). To get the line A, a line was arbitrarily drawn on a semilogarithmic paper It was then considered what would occur if the aggregation was double so fast, that means that the platelets were more sticky and then you get the line B You can also consider what would happen if the aggregation was reduced by half and you get line C The corresponding changes can also be accomplished by changing the speed of the recorder You can also consider what would happen if not all particles or platelets are aggregating. To get the line D a proportion of the platelets are not aggregating, in this case it would in reality be

$8 = 2^3$ (1)^a



$27 = 3^3$ a^a

Figure 1

Light passage through particle suspension of one layer randomly distributed in 3 levels

T ble I

Formula for light passage through particle suspension.

$$y = \frac{(a-1)^{a.n}}{a^{a.n}}$$

Proportion of light beams transmitted w thout absorption.

= pos bility of position in different levels.

= numbe of particle (layers).

$$\log \frac{1}{y} = c.n$$

Table II

Rate of aggregation

$$n = \frac{1}{t} \quad c$$

Number of aggregates is inversely proportional t time

Table III

Equation of primary ve of platelet aggregation

$$\log 100 \quad \log y = \frac{c}{t}$$

y = transmission

t = time

= onstant

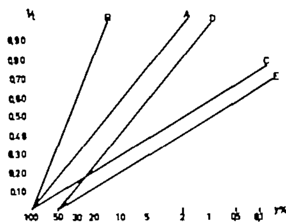


Figure 2

Lines on semiloga thmic paper constru t d according to the theoretical model f platelet aggregation (See text)

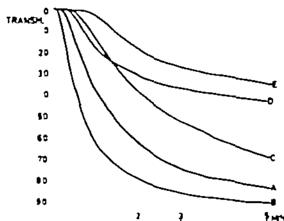


Figure 3

Lines of l gure transferred t ordinary scale

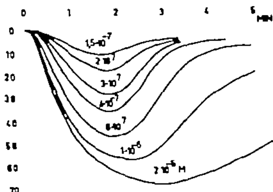


Figure 4

Platelet aggregation curves after addition of various doses of ADP to citrate PRP

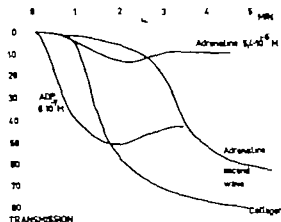


Figure 6

Aggregation curves of ADP adrenaline and collagen in citrate PRP



Figure 5

Curves of Figure 4 transferred to semilogarithmic paper according to theory

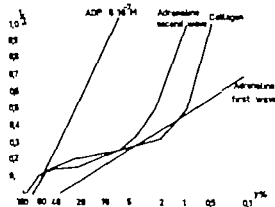


Figure 7

Aggregation curves of Figure 6 transferred to semilogarithmic paper

about 18% of the platelets. If you changed the sensitivity of the recorder a similar change will occur. If you reduce both the rate of aggregation and the number of aggregating platelets you will get the line E. On a semilogarithmic paper the slope of the curves indicates the degree of stickiness, whereas the intersection of the -axis is the point the aggregation would have reached if it had gone on for endless time and it corresponds to the number of platelets that do not aggregate. If for instance this intersection occurs at a transmission of 50% you can mix platelet-poor and platelet-rich plasma until you get this transmission and you will find that the mixture consists of about 20% platelet-rich plasma, which indicates that 20% of the platelets do not participate in the aggregation.

Figure 4 shows platelet aggregation caused by various doses of ADP. The interpretation seems difficult, but if we transfer the curves to semilogarithmic paper according to the equation earlier given straight lines will be found (Figure 5). As you can see the main differences are due to a different number of activated platelets that aggregate, i.e. the intersection of the x-axis, but also that with increasing concentrations of ADP there is a moderate increase

in the stickiness.

In figure 6 aggregation by ADP adrenaline and collagen are given and also the second wave of aggregation by adrenaline In figure 7 you see that the primary wave of ADP and adrenaline induced aggregation corresponds to this equation but this was not the case for collagen -induced aggregation and the second wave of adrenaline-induced aggregation where there is a continuous increase in the number of activated platelets and the degree of stickiness. The fact that the primary wave of aggregation by ADP and adrenaline satisfies the equation indicates that a limited number of platelets have been activated and that everything then goes on without any other change until the state of activation disappears. There will then be disaggregation with a rapid burst and liberation of free platelets.

Figures 8 and 9 demonstrate the influence of different temperatures and show that you can use lower doses of ADP to activate the platelets at a low temperature whereas on the other hand, the degree of stickiness is increased at a

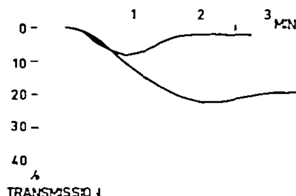


Figure 8

Aggregation of platelets by 10^{-6}M ADP in citrat PRP that had been kept at 37 C respectively 0 C

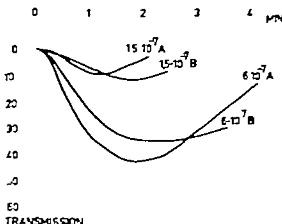


Figure 10

Aggregation by various doses of ADP in citrate PRP immediately after preparation and after storage for 2 1/2 hours.

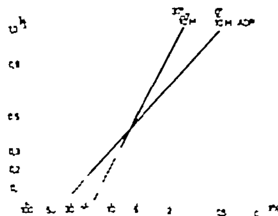


Figure 9

Aggregation curves of Figure 8 transferred to semi-logarithmic paper

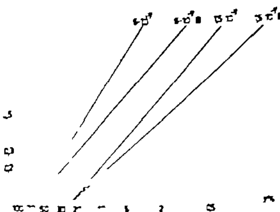


Figure 11

Aggregation curves of Figure 13 transferred to semi-logarithmic paper

higher temperature The platelets then aggregate faster but the number of activated platelets is less and they are activated for a shorter time so that disaggregation starts earlier

In figure 10 the effect of storage of the plasma for two and half hours was investigated and the interpretation in figure 11 demonstrates that the intersection of the x-axis is unchanged and therefore also the number of aggregating platelets, but that the stickiness was decreased which is indicated by the flatter slopes. The increased duration of the activated state will produce the longer aggregation curves.

REFERENCE

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in the stickiness.

In figure 6 aggregation by ADP adrenaline and collagen are given and also the second wave of aggregation by adrenaline In figure 7 you see that the primary wave of ADP-and adrenaline-induced aggregation corresponds to this equation but this was not the case for collagen -induced aggregation and the second wave of adrenaline-induced aggregation where there is a continuous increase in the number of activated platelets and the degree of stickiness. The fact that the primary wave of aggregation by ADP and adrenaline satisfies the equation indicates that a limited number of platelets have been activated and that everything then goes on without any other change until the state of activation disappears. There will then be disaggregation with a rapid burst and liberation of free platelets.

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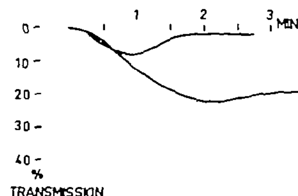


Figure 8

Aggregation of platelets by 10^{-7} M ADP in cit te PRP that had been kept t 37 C respectively 0 C

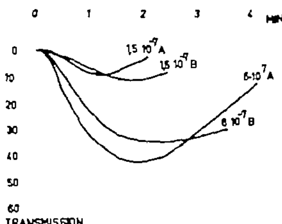


Figure 10

Aggregation by various doses of ADP in citrate PRP immediately after preparation and after storage for 2 1/2 hours.

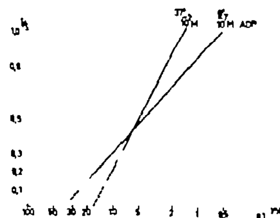


Figure 9

Aggregation curves of figure 8 t ansferred to semi-logarithmic p per

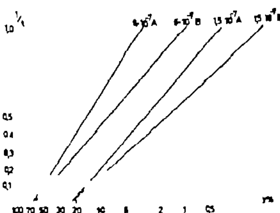


Figure 11

Aggregation curves f figure 13 transferred to semi-logarithmic paper

DISCUSSION

J.R. O BRIEN I am impressed by the in genuity of Professor Born s centrifugation ex periments and I must accept that the inulin is there among the platelets. But in my experience if unfixed platelets are centrifuged and then fixed and examined by EM they always show tight-fit mosaic apposition with no suggestion of spaces between the platelets. Yet you suggest that the platelets are spiky and could not fit together This seems a very special situation. Can you comment ? Does EDTA keep them apart, spikes and all ?

G V R. BORN You can do it with inulin but, if you want to be less unphysiological, you can use ^{125}I or ^{131}I-labelled plasma albumin the results are essentially the same Neither inulin nor albumin is adsorbed on the platelets. The question is whether under our conditions, the platelets are packed so tightly by centrifugation that they cannot be packed any tighter Maximal tight packing seen by electron micro-scopy probably occurs only when the second phase has started. The microspikes and pseudo-podia-like protrusions are presumably so rigid that they are not bent under the centrifugal force we use but, even if they do their presence would still increase the extracellular space

P.M MANNUCCI Among the factors influ-encing the optical density test, I would like to stress the importance of controlling the tem-poral drift between collection of the blood, preparation of PRP and the performance of the optical density test If one looks at the descrip-tion of the methods in the various published papers, it is not often said by the authors whether they take care of keeping constant the intervals between the collection of the sample the preparation of PRP and the performance of the aggregation test We have seen that the reactivity of the platelets to the aggregating agents passes through various phases. Within the first 30 minutes after the preparation of PRP they are weakly sensitive to ADP or any other clumping agent in the sense that one gets a lower increase in transmittance Then, between 45 and 90 minutes, there is a phase of maximal reactivity and then again a phase of decreased reactivity This has been seen by us and also by Dr Silver with another method in the United States. Another phenomenon which is quite influenced by the temporal drift between the collection of the blood preparation of PRP and the performance of the experiments, is the occurrence of the second wave of ADP or adrenaline-induced platelet aggregation. For instance when an inhibitor of platelet function is tested control platelet-rich plasma added with buffer is usually tested first followed by different concentrations of the substance under investigation A long period of time elapses in the meanwhile and the degree of inhibition or of aggregation might well be influenced by the temporal drift Therefore we feel that in order to obtain a correct pattern of results the control PRP has to be repeated several times throughout the whole set of tests. I might be wrong but I do not think that it has been said often enough in the methodological part of papers on platelet aggregation that the time between collection of the blood preparation of PRP and performance of the optical density

test has to be kept constant.

Dr Born commented on our paper by Dr Sharp and myself on platelet volume and on the influence of aggregating agents and inhibitors. He says that what we measured and Dr Zucker also measured might be variation in shape rather than in size His experimental evidence is quite convincing but on the other hand I think that the principle of the Coulter Counter is such that you cannot measure anything other than volume In our original work we tried to correlate the variation in volume as measured by the instrument with variation in shape doing phase contrast microscopy and then electron microscopy As far as I know about the physical principle of the Coulter-Counter there is evidence that volume and not shape is the relevant factor in fact it is the displacement of the fluid by the particle and the subsequent decrease in conductivity between the two electrodes which gives the height of the impulse and I do not see how shape could influence this phenomenon

G V.R. BORN Could I reply to Dr Mannucci? First measurement of volume by measuring size under the microscope whether light or electron microscopy is obviously difficult because you measure cube-roots of what you want to know It is in principle much more accurate to measure the volume of something directly than by its cube-root On electron-micrographs you also have to measure in different directions and you have to make very many measurements and treat them very carefully The other problem is the Coulter Counter needless to say we have thought a great deal about this. The Coulter people tell one that its principle is that the impedance is proportional only to cell volume but actually that needs not to be the only thing that influences the impedance Consider what is happening A stream of fluid rushes through a little hole Platelets are discs and presumably they orientate themselves in the stream with the long diameter parallel to the stream-lines this will cause a certain impedance Now after the change in shape a platelet is no longer a flat disc and it is easy to see that when it passes through the hole even without a change in volume there will be a change in impedance I am sure something like this must be the explanation.

P.M MANNUCCI Certainly this sounds as an interesting idea On the other hand, the explanation of the displacement of the conductive fluid given by the Coulter-people seems to me rather convincing. I think to have given the impression that we wanted to measure volume with the phase contrast and electron microscopy No we used that only to watch the shape and of course we know that one cannot measure volumes with these methods.

A.A. SHARP I think I wish to take exception to Professor Born's hypothesis in relation to cell size and shape The cell is passing not through a hole as you described it but in actual fact a small tunnel It therefore measures volume of fluid displaced by the cell in this tunnel and this is not dependant on shape but volume only I think that this principle has been applied and proved for red cells and many other particle sizes and shapes in industrial research. I think I am right in saying you cannot blame Coulter's for claiming 'that their equipment measures volume and not shape as the physicists have worked on this and the evidence is that it is total volume which causes the proportional impedance or resistance to the current. I think your observations on your thrombocrit are perfectly valid but I do not think it is quite right to say that because thrombocrit gives one set of results and the Coulter gives you different information, one rules the other out I think you are measuring two different things, and the artefact produced by high speed centrifugation could, in a vital thing like a platelet change its shape If one calculated the mean cell volume of the platelets in your thrombocrit and correlated this with the Coulter I just wonder whether in fact they might not show still an increase in volume

G V R BORN First I do not blame anyone

these are scientific observations. Secondly the analogy argument from red cells and particles from other systems does not hold Every system or particle has to be considered apart if one wants to provide the most reasonable explanation of ones results.

P.M. MANNUCCI If I am right in under standing, Dr Born you say that as a positive control for the increase in volume you used in your system a hypotonic solution. I wonder whether you are prepared to consider something we did in our paper as a positive control in our system. We studied the effect of cocaine and this agent gave according to the Coulter a very marked increase in volume as well as in shape Do you think that could be a sort of positive control comparable to the one you are suggesting ?

G V.R. BORN I have no idea one has to do the experiment What effect were you anticipating with cocaine ?

P.M MANNUCCI It might be an experimental model for swelling similar to that of the hypotonic solutions which you have made

G V.R. BORN We could simply do the experiment and see

J CAEN I apologize if my question is not related to the London-Oxford match but to the remarks by Dr O Brien We have noted for a long period of time that in many cases we lose a part of the platelets during centrifugation and I was very interested to see that Dr O Brien, using the optical density method, has not found differences in the reactivity of those platelet rich plasmas which had lost about 30 per cent of the platelets and those which had lost nothing So the question arises has he lost for instance the heaviest platelets and not the lightest ? I suppose that the lightest could be those which are of a very great importance for the estimation of the optical density change

J R O BRIEN I cannot really answer this except to say that I compared those plasmas in which I had lost many platelets with those in which none were lost and I could not find any difference in a large series of tests. However it is obviously possible even probable that you are losing a special group of platelets.

R. GROSS I would only like to confirm Dr Born s statement. We are unable to make any measure of the real size in preparations fixed by light or electron microscopy Some years ago there was an extensive study about this question in the Marburg anatomical institute and the results were that this size was dependent on the velocity of fixation on the kind of fixation medium and of course on the original size so that you cannot say anything about the real size by measuring the diameter in fixed prepara tions.

J.R O BRIEN At the Miemo meeting I reported platelet size from fixed prepara tions (1) Your remark is probably true but a relative size difference should still be valid

R. CROSS That is in some sense right in relation to the relative size as you say but the most important feature of these examinations in my opinion was that there was not only a corresponding shrinking but that there was shrinking depending on the original size so that the relations between the different cells were changed too

J.J SIXMA I would like to show two slides that show the influence of the osmolarity during glutaraldehyde fixation The first slide (Figure 1) shows the effect of hypertonicity caused by sucrose (1 per cent glutaraldehyde in 0.07 M phosphate buffer sucrose added to a final osmolarity of the mixture of about 500 mosmole) The surface connected tubules are dilated This dilatation is absent when fixing at 280 mosmol. (1 per cent glutaraldehyde in 0 07 M phosphate buffer (Figure 2)). I think *it is* of importance to realize that the osmolarity of the *fixation fluid still* influences the electron microscopical picture during at least 20 minutes of fixation



Figure 1

Platelet fixed in 1 per cent glutaraldehyde, 0,07 M sodium phosphate buffer pH 7.40 and sucrose added to a osmolarity of the PRP/fixative mixture (1 4) of 500 mosmole Osmium postfixation Uranylacetate and lead citrate staining. Dilatation of the surface connected tubules is apparent.

H REUTER I would like to refer to the question of Dr Mannucci as to the time when the tests are done after preparation of PRP Immediately after preparation we have another curve than 285 minutes later The plasma was stored at room temperature You see that the two phases of aggregation change

J.R O BRIEN I have a question for Dr Mannucci In this temporal change in the platelet responsiveness, was this due to time elapsed before centrifugation or after when the platelet-rich plasma was standing on the bench ? The second question is what was the degree of change that you found with time Was it 5 per cent or 50 per cent increase in reactivity ?

P.M. MANNUCCI This refers to variations in platelet-rich plasma which has been processed as soon as possible after the collection of the blood. I am sorry I have not taken with me the actual data so I cannot show them to you but it was indeed quite a large variation. I would like to hear from dr Hardisty about this topic because I think that he has got some evidence of a marked variation in the capacity of ADP to elicit the second wave of platelet aggregation related with the time from the collection of the sample to the performance of the test I think that the lack of controlling the constancy of the interval between preparation of platelet-rich plasma and the actual performance of the optical density test might well explain the absence of the second wave of ADP or adrena-



Figure 2

The same platelet-rich plasma as in figure 1 as fixed with 1 per cent glutaraldehyde in 0 07 M sodium phosphate buffer pH 7 40 and sucrose added to osmolarity of the PRP/fixative mixture (1 4) of 280 mosmole Further procedure as Figure 1 The surface connected system is present but not dilated. Most platelets have retained their disk shape.

line-induced aggregation as found by several authors. We feel that a multichannel aggregometer and recorder is really needed when several samples of platelet-rich plasma are to be compared fo their aggregation patterns in the same experimental conditions I wonder whether Professor Born could devise something like that I am sure that would be a great advance

R.M HARDISTY The reason why I cannot in fact answer Dr Mannucci's question is because I have a multichannel aggregometer and we have deliberately avoided studying the effect of time on this phenomenon by taking it from the other point of view and trying as far as possible to eliminate variation in time in our studies. In fact by the use of a six channel aggregometer which is in fact six separate aggregometers linked to a multichannel recorder we have attempted to confine the time of testing be tween one and two and a half hours from the time of obtaining blood from the individual.

G V.R. BORN We have tried six-channel recorders Dr Hardisty has done so very successfully We came to the conclusion that it would be better to make a small cheap one channel recorder of which one can use six in parallel and this is what we have now done In the new aggregometer as little as 0._ ml of

Question N 3

WHICH PHYSICAL OR CHEMICAL ALTERATIONS OF THE PLATELET SURFACE ARE PROVOKED BY DIFFERENT AGGREGATING SUBSTANCES ?

DISCUSSION

R. GROSS
J HUGUES
G V.R. BORN

J R. O'BRIEN
E.F LUSCHER

9 PLATELET FACTOR 4 (PF 4) AND THE PLATELET MEMBRANE

J R. O Brien

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One of the important changes that occur during aggregation may be the development of PF 4 or heparin neutralizing activity (HNA) on the surface of the platelet immediately after adding ADP (1) If ADP is added to citrated PRP at 37°C and mixed but not stirred, and this mixture is immediately tested for HNA with minimal shaking until near the clotting time It is shown that moderate HNA develops. Stirring or even pipetting causes some aggregation and much less HNA is demonstrable This HNA is spun down by gentle centrifugation just sufficient to spin down most of the platelets so it is presumably on the membrane

This ability to neutralize heparin with its negative charge very strongly suggests that the platelets, when they become sticky under the influence of ADP have developed positively charged sites. But these sites developed equally in Glanzmann platelets which are totally non-sticky This at least means that surface HNA is not the only determinant of stickiness Indeed there is no evidence that it has a direct effect on stickiness. Adrenaline makes platelets sticky but platelets so treated do not develop surface bound HNA

Some of the events following the addition of ADP relevant to the membrane may now be summarised. There is a shape change which suggests a change in membrane or sub-membrane (e g. microtubules) constituents. At variance with Born I still think there may be a change in volume in which case water must enter indicating a change in membrane permeability The platelets become sticky but we don t know what this means. They also develop HNA which strongly suggests a decrease in negative charge As a quite unproven speculation I wonder if this involves a change in mucopolysaccharides. When aggregation (one form of propinquity) begins, the contact seems to send a message from the membrane to start the release mechanism. The passage of the various materials released surely indicates another change in membrane permeability PF 4 is also released at this time and Poplawski and Niewiarowski have reported (2) that it is a low molecular weight glycoprotein or polypeptide According to our studies PF 3 the phospholipid particles, separate only later and some PF 3 activity also remains exposed on the platelet membrane Each one of these changes must indicate some profound reorientation of membrane molecules or a complete change in the chemical or biophysical properties of the membrane

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10 STRUCTURAL CHANGES OF PLATELETS IN CONTACT WITH SOME AGGREGATING AGENTS

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University of Liège, Belgium

We have studied the structural changes of platelets exposed to aggregating agents prior to the occurence of aggregation.

Under these conditions one sees (in approximately 1/5 of platelet sections) very characteristic racemose formations, which we have termed microvesicular clusters (MVC).

Each cluster varies from 0.5 to 1 micron in diameter and consists of a rounded evagination of the platelet membrane which surrounds numerous tightly-packed spherical vesicles. These microvesicles, about 150 millimicrons in diameter are each bounded by a double-layered membrane They have no discernible contents. No more than one such cluster has ever been observed in any platelet section The MVC is most often located at the end of a pseudopod (Figure 1) occasionally it occurs as a herniation extruding from the surface of the platelet. However it may also be lodged inside a large vacuole within the platelet itself in which case the MVC membrane is continuous with that of the vacuole

After contact with collagen the MVC may contain dense particles which are identical in every respect to the glycogen granules located in the hyalomere These particles can reach impressive proportions in the clusters (Figure 2) at a time when glycogen is becoming noticeably less abundant in the remainder of the platelet. It may be that intracellular glycogen is eliminated from the platelet via the microvesicular cluster

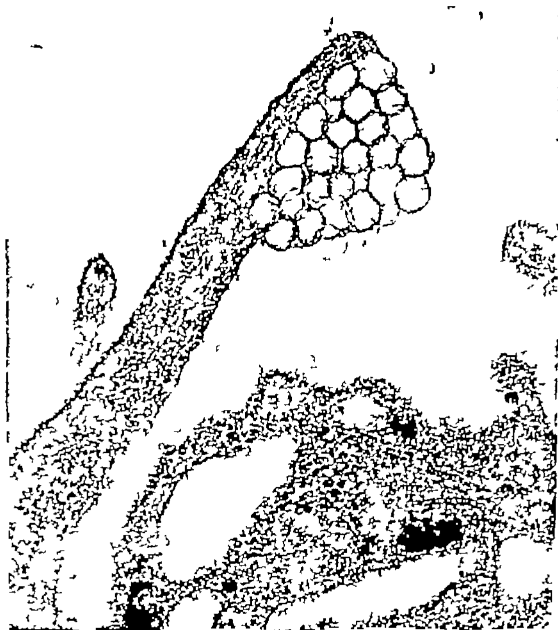


Figure 1

A microvesicular cluster located at the end of a pseudopod. Final magnification 87 000 X (Electron micrograph taken by Dr L. J. Simar, Institut d'Anatomo-Pathologie (Prof E.H. Betz), University of Liège)



Figure 2

Dense particles in microvesicular cluster. Final magnification 81,250 X. (Electron micrograph taken by Dr. L. J. Simar, Institut d'Anatomo-Pathologie (Prof. Dr. E.H. Betz) University of Liège).

DISCUSSION

R. GROSS I have a question to Prof Hugues have you any idea about which form of glycogen (that contained in the microgranules or that diffusely present in the platelet) is changing in the way you showed us ?

J HUGUES I think only the glycogen in the granules, but I do not yet have evidence for that.

G V.R. BORN Like Holmsen I am much intrigued by the analogy of platelets with muscles. One has to assume that the ADP interacts with some protein constituent of platelets, presumably in some similar way to that with which it interacts in muscle One does not know what the binding sites are in the amino acid chains of muscle proteins, but something is known about how ADP interacts with actin in its different forms. ADP interacts with G actin reversibly and exchanges very rapidly On the other hand with F actin the ADP is slightly bound. I think that one of the crucial questions is the same for muscle and platelets, namely the chemical nature of the receptor site for ADP One wants to know the three-dimensional configuration of the part of the protein with which the nucleotide interacts.

J.R O'BRIEN You have been drawing an analogy between muscles and this platelet membrane If I had to guess at the present stage I would suggest that mucopolysaccharides and glycoproteins in the membrane are involved. Can someone tell us what is known about glycoproteins and mucopolysaccharides in muscle ?

E.F LUSCHER To my knowledge there is no cell membrane which does not contain mucopolysaccharides platelets in fact seem to contain them in comparatively large amounts.

G V.R. BORN The mucopolysaccharides have different components, including blood group substances and others which are present also on the red cells.

Question N 4

ROLE OF THE RELEASE REACTION IN PLATELET AGGREGATION

11 H. HOLMSEN and H.J DAY The Platelet Release Reaction and its Role in Platelet Aggregation.

DISCUSSION

J HUGUES
H HOLMSEN
J.R. O BRIEN
G V.R. BORN
S BYGDEMAN
E.F LUSCHER
J CAEN
S CRONBERG
J.J SIXMA

11 THE PLATELET RELEASE REACTION AND ITS ROLE IN PLATELET AGGREGATION

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Some scientists share this view the platelet release reaction consists in extrusion of those materials being located in either the dense bodies or the α granules. If one could stick to this definition and let the term platelet release reaction" mean only this, much semantic disagreement among scientists would disappear. The main reason for not accepting this definition of the release reaction might be that during nearly all conditions under which the platelet release reaction occur there is some unspecific platelet lysis. For example platelets having been incubated with radioactive adenine adenosine or orthophosphate (2) always liberate small amounts of *radioactive* nucleotides in add tion to the high amount of nucleotides being *released* during release reac tion nucleotides which are *non-radioactive*. Another striking example on unspecific lysis is that platelets from patients lacking stored ADP and ATP do aggregate with collagen (7) Here it is the appearance of labeled extracellular ADP originating from the metabolic active pool in platelets which probably was responsible for the aggregation. Another reason for the apparent unwillingness to accept the above definition of the release reaction might be the different ways by which the release reaction is induced and inhibited. The main difference in way of action among different release inducers lies probably only in the "induction step" We have subdivided the release reaction into three steps (6) a first step (induction) which is the interaction between the platelet membrane and the release inducers a second step (intracellular transmission) in which an impulse or a substance created during the induction is transmitted from the membrane and to that locus in the cell where ATP energy is utilized for the third step extrusion of granular material through the outer membrane or the canalicular system (this extrusion step consists in relative movement between granula and platelet membrane towards each other fusing at the point of contact and finally a forceful extrusion of the granula content to the extracellular medium) The two last steps, intracellular transmission and extrusion, appear to be *common for all release inducers*. Many inhibitors of the release reaction also interfers with the induction whereas some clearly work on step 2 or 3 (2,6). Our main reason for using the term "release reaction" for the platelet response to *all* the different release inducers is that *they cause release of only those substances which are contained in the platelet granules (4)* irrespec

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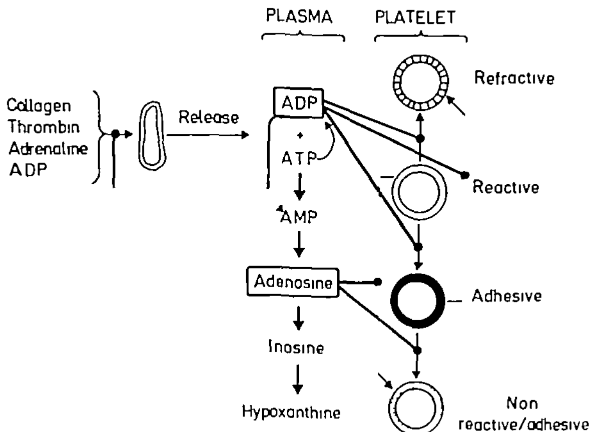


Figure 1

The post release adenine nucleotide platelet plasma situation. For explanation, see the text and ref 5

and Rozemberg, this Conference)

Of the ATP which is released, only 10% is converted to ADP the rest is directly de phosphorylated to AMP which is again rapidly transformed to adenosine (5) Adenosine is a potent inhibitor of platelet aggregation and makes reactive platelets non-reactive or non-adhesive also a platelet which is in an aggregate might turn into a non-reactive platelet. ATP AMP inosine and hypoxanthine have no *direct* effects on platelets, except perhaps in inhibition of swelling as Born showed earlier to-day The last effect we could also attribute to ADP is to amplify the release reaction since ADP in itself is a release inducer

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DISCUSSION

J HUGUES I have a question for Dr Holmsen do you think that the action of collagen is mediated by ADP ?

H HOLMSEN I think most of what we see in the aggregometer when the platelets aggregate is mediated through ADP but I do not think at all that the first small aggregates which form are ADP-mediated.

J.R. O BRIEN May I make a comment on Dr Holmsen s remarks. He said that after the addition of ADP platelets became non-responsive to ADP and this might explain the disaggregation. I can not deny this possibility but it is difficult to reconcile with some old observations of mine (3) If you add a large quantity of ADP to unstirred PRP the platelets remain sticky in the sense that they will aggregate if you stir them after 20 or 40 minutes but at the end of an hour these platelets are totally non-responsive to ADP They are no longer sticky although active ADP is still present However if you add adrenaline then these platelets will stick, and conversely if you incubate platelets but do not stir them with adrenaline at the end of an hour these platelets are then no longer responsive to adrenaline but will respond to ADP These are the facts, I do not know the explanation one possibility is that stickiness persists when there is disequilibrium of ADP across some membrane and by one hour complete equilibrium has been achieved.

G V R. BORN David Mills in my department has similar results to Holmsen s What interests me is whether the release reaction can account for the physiological response I would very much like to hear what Holmsen thinks about that.

H HOLMSEN I admit we do not have any direct proof of the importance of the release reaction *in vivo* Bygdeman was mentioning some experiments with laser-induced trauma and he showed that the number of thrombi was not affected by treatment with release inhibitors I am not so sure if these experiments are convincing Under this laser beam-induced trauma will you not have enough ADP liberated from the tissue to primarily make these aggregates ?

S. BYGDEMAN If you use ADP inhibitors in this *in vivo* model for instance adenosine or dextran you can inhibit platelet aggregation at the site of the endothelial trauma but not if you use pure release inhib tors

H HOLMSEN Do you not think that the way you have made this trauma liberates so much tissue ADP that this accounts for the aggregation, that the release reaction would not have anything in addition to do with this sort of accumulation of platelet plugs ?

S BYGDEMAN Yes it is possible

G V R. BORN I have something to contribute here which may perhaps be useful. We have already looked at the effect of some inhibitory substances on the first order rate constant of white body formation which I showed you

earlier this morning. We infuse the inhibitor into the animal's jugular vein. We determine the rate constant before and after the inhibitor and while it is being infused. Unsurprisingly adenosine causes the rate constant to be decreased. Because we were interested in the question whether the release reaction is involved *in vivo* we have also infused aspirin on the assumption that aspirin inhibits release but does not do anything else. I do not know whether that assumption is right. Anyway the answer is that aspirin does in fact diminish the growth rate constant of these white bodies. Holmsen will be pleased about that.

E.F. LUSCHER Since 1947 it has repeatedly been proved that in a normal animal the formation and fragmentation of a white thrombus continues for hours. It is difficult to visualize how something originating from the vascular wall could promote aggregation for such a long period. No doubt the essential stimulus comes from the platelets themselves and most likely it is material released from them which is responsible. I think one has to be quite careful about studying inhibitors unless they act irreversibly such as aspirin, otherwise the concentration of the inhibitor in the interspaces between platelets as compared to the concentration of material released from them may be quite different from a system containing evenly suspended platelets and defined inhibitor concentrations.

J. HUGUES I would like to add one word in agreement with what Dr. Lüscher just said. In an old experiment when we cut a microvessel we found that it was possible to inhibit formation of the hemostatic plug with citrated washing fluid for a long time. When we again used normal washing fluid plug formation occurred very rapidly. It is very difficult to postulate that something can be released over such a long period of time from a small vessel wall.

J.R. O'BRIEN Professor Mitchell made an important point recently at a meeting when the white bodies made in the rabbits' small vessels were discussed. He emphasised that if the white bodies embolise and then get stuck they will disintegrate. This is in contrast to the observation that a white body grows while it is in contact with the damaged area. He raised the point of some transmitter substance coming from the damaged area.

E.F. LUSCHER Spontaneous embolisation is always observed when such a thrombus forms. In fact it is a process which is inherent in the formation of platelet thrombi in normal animals. Pickering has made a movie picture of the background of the eyes of patients with severe atherosclerosis, which shows the formation of platelet emboli to be a permanent process. It appears as if the fragmentation of thrombi which never get consolidated is something which is almost a physiological process.

J. CAEN Just coming back to the question of Dr. Holmsen, I believe that in some constitutional or acquired haemorrhagic disorders, in which the release in the presence of collagen or epinephrine is absolutely nil or very diminished, the ADP-induced aggregation is strictly normal or at least subnormal, so it is quite possible that ADP comes from the platelet but possibly from other cells, or from endothelial cells. In Bygdeman's experiment it is tempting to postulate that a normal activity of extrinsic ADP can occur although the intrinsic ADP is unable to act due to its low concentration in the releasable pool; still these platelets respond quite normally to extrinsic ADP wherever this comes from.

J. HUGUES Is there any evidence that ADP is released from the endothelium?

J. CAEN I do not say that ADP comes from the endothelium. I say that it is not coming from platelets but from elsewhere.

H. HOLMSEN I did not quite get Caen's statement. I thought that these patients who lack the pool of nucleotides had quite a

tendency to bleed which should be a good proof that the release reaction is important, in normal haemostasis at least

J CAEN I agree with this. What I say is that this type of platelets responds normally to ADP I agree that these patients have a bleeding disorder

G V.R. BORN About the question of whether something is continuously released from a site of injury I do not see the difficulty is it really necessary that something has to be released ? At an injury site in which collagen or procollagen or basement membrane or whatever is exposed, a certain proportion of passing platelets make contact with the site Thus contact induces the primary event at the platelet membrane which causes adhesion and a little later presumably release It is really necessary to postulate that something has to be released from the injured vascular wall ? Is it not enough that some of the passing platelets are statistically certain to hit the site ? With any kind of injury and removal of endothelium and exposure of collagen some platelets will touch there And when they touch they change Is that your idea, Professor Hugues ?

J HUGUES Yes of course in contact with collagen or basement membrane

S CRONBERG When I was working with Dr Caen and his collaborators, we made some investigations on the release in washed platelet suspensions. We then found that different inducers had different actions. Thus a particle suspension like kaolin latex particles or collagen released ADP even in a weak EDTA solution and without any cofactor (1) Nevertheless, at a low temperature calcium had a marked potentiating effect and at a higher temperature calcium was necessary for the release of insoluble enzymes such as platelet acid phosphatase This particle-induced release from washed platelets was not inhibited by acetylsalicylic acid.

On the other hand, ADP and adrenaline did not induce release unless the platelets first had aggregated Besides plasma had to be added to provide a necessary cofactor different from fibrinogen (2) This release by ADP or adrenaline was inhibited by acetylsalicylic acid We therefore concluded that the release by particles was secondary to adhesion or phagocytosis and was mediated by a different mechanism than release by ADP and adrenaline which was secondary to the formation of large aggregates inside which the internal milieu was quite different and special reactions occurred.

J.J SIXMA I would like to ask Dr Holmsen if he feels that acid hydrolases and fibrinogen can be present in one granule Is it possible for fibrinogen to be in the same granule with cathepsin ? Do you suppose that there exist two types of alpha granules ?

H. HOLMSEN I am not the right one to answer that question Of course there might be two types of granules between which we cannot distinguish but I do not see why the acid hydrolases should not exist together with a protein because at the physiological pH 7 4 most of these acid hydrolases are inactive (and, actually we do not know the pH inside the granules)

S BYGDEMAN I would like to make a short comment concerning the results obtained with the laser-beam technique The lack of effect with acetylsalicylic acid on platelet aggregation in vivo should not be interpreted to mean that the release reaction does not play a role in thrombus formation or on the formation of a haemostatic plug since in this model you can only study the initial phase of thrombus formation What I wanted to stress was *that the* release raction did not appear to play a role for the immediate platelet-endothelial interaction.

J HUGUES I should like to ask a question what about *the kinetics* of the release reaction ? *I* ask this question because in our laboratory we have found some evidence that *during the lag* period after exposure to collagen

PF3 is made available before aggregation occurs.

H HOLMSEN This is difficult to answer because of the lysis phenomenon I have discussed and which I think is responsible for the same amount of factor 4 which is liberated very early actually *before* you can see any aggregation For example if one is using collagen, it is of importance how the nature of particles is. Using soluble collagen a sort of "random fibril" is formed instantaneously in plasma and it is very hard to speak about kinetics in this uncontrolled system. We have not extended our collagen studies further than using a very high dose of collagen in order to assess what is released and retained. So far it is very much the same as the thrombin-washed platelet system. I might also add that we have repeated all our enzymes which I showed you here in a native PRP system which clotted. It is exactly the same discrimination between the granule located enzymes and the soluble-mitochondrial-membrane located enzymes. The three latter ones are completely retained during clotting, whereas those located in the granules are released to an even greater extent than in the washed platelet-thrombin system (*J Lab. clin. Med.*, in press, 1971)

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Question N 5

HOW IS PLATELET AGGREGATION LINKED WITH INCREASED AVAILABILITY OF PLATELET FACTORS 3 AND 4 ?

DISCUSSION

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J.J SIXMA
S CRONBERG
J W ten CATE

A. SHARP
J CAEN
P.M. MANNUCCI
J.L. DAVID
H. HOLMSEN

12. PLATELET FACTOR 3 AVAILABILITY AS AN IRREVERSIBLE CHANGE OF THE PLATELET MEMBRANE

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By aggregating platelets in platelet-rich plasma (PRP) with ADP and then reversing the aggregation by means of promethazine or imipramine one can make platelet factor 3(PF3) available in PRP containing free unaggregated platelets If this PRP is then centrifuged at various speeds, in order to bring down varying proportions of platelets, and the Stypven time of the supernatants determined as an index of PF3 activity it can be shown that most of the activity remains associated with the platelets themselves, and only about 12 % is released in finely particulate or soluble form into the plasma (1). The most likely interpretation of these findings is that PF3 availability represents a platelet surface change and that this change is dependent upon aggregation, or what O'Brien has called propinquity it is certainly not brought about by ADP in the absence of aggregation as can be shown by adding ADP to PRP without stirring, when very little change in Stypven time occurs. On the other hand if platelets are brought into contact with each other by simple centrifugation of citrated PRP without the addition of any aggregating agent, and are then resuspended in their own plasma, the Stypven time is markedly shortened (Figure 1), and this effect is enhanced with repeated centrifugations.

In summary I would suggest that PF3 availability is an irreversible change produced in the platelet membrane surface by contact with either glass or other foreign objects or other platelets. What the nature of this change is, I really do not know I should like to suggest, as a working hypothesis, an altered reactivity with plasma clotting factors, possibly a state of increased affinity for absorbed clotting factors,

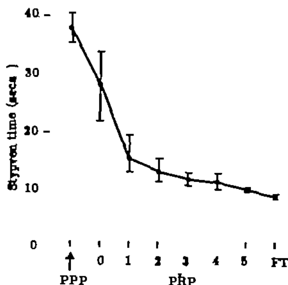


Figure 1

Effect of centrifugation and resuspension of platelets on the Stypven time.

so that one has an essentially physical change leading to an active catalytic surface on which clotting factors can react together in optimal conditions.

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13 PLATELET FACTOR 3 (PF 3) AND PLATELET FACTOR 4 (PF 4) AS A GUIDE TO PLATELET MEMBRANF STRUCTURE

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Platelet factor 3 may be said to exist when a platelet membrane or material derived from a platelet causes shortening of the Stypven time It indicates that some phospholipid molecules (PL) are available and cause shorter clotting times. This PL can exist in three forms

1 Bound and inactive in the membrane This occurs in normal, non-sticky unactivated plate lets.

2. Exposed on the surface of a platelet (2) If fine glass beads are added to platelet-rich plasma (PRP) and immediately a Stypven time is carried out on this mixture the clotting time is shorter than that before the beads were added. If the glass beads are allowed to settle with their attendant platelets stuck to them, then the Stypven time is almost as long as in untreated PRP Thus it is the platelets stuck to the glass that initially have exposed PL on their surface Hardisty has also just given other evidence of platelet bound PL activity

3 PL can exist as particulate material separate from the platelets. For example after 30 seconds of exposure to glass in the experiment mentioned above it is found that the super natant, after centrifuging platelets and glass beads, has PL activity It is also exposed and released later in the normal release reaction.

PF 4 is said to exist when a platelet membrane or material derived from it has the power to neutralize heparin and so shorten a heparin-thrombin clotting time This heparin neutralizing activity (HNA) also can exist in three forms

1 Bound and inactive in the membrane This occurs in normal non-sticky unactivated platelets.

2. Exposed on the platelet surface If ADP is added to non-stirred PRP then these sticky platelets, providing they have not stuck together have moderate amounts of HNA (3)

3 Soluble HNA is released from platelets during the release reaction and is found in serum (1)

These two types of membrane upset can occur together in hypotonic osmotic damage freezing and thawing or late in the release reaction. PL is exposed alone when platelets are sphered by adding lypophilic molecules like cocaine or when they are first stuck to glass beads. HNA only is exposed when platelets are made sticky by adding ADP Aspirin inhibits HNA but has no effect on PL (4,5)

These observations at present shed no light on membrane structure but any concept of structure must be consistent with these observations.

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14 PLATELET FACTOR 3 AVAILABILITY AND THE RELEASE REACTION

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The platelet factor 3 (PF 3) availability is overestimated when the shortening of the Stypven time is used as a measure. Clotting time and percentage PF 3 are in a strictly rectilinear fashion related when plotted on double logarithmic paper. In this way clotting times can be converted to percentages using a dilution curve of four times frozen and thawed platelet rich plasma of the same individual (Figure 1).

Although this is certainly an artificial way of preparing "total PF 3" we feel that it has some bearing on the capacity of the platelets to make phospholipid available and it is an easy way to

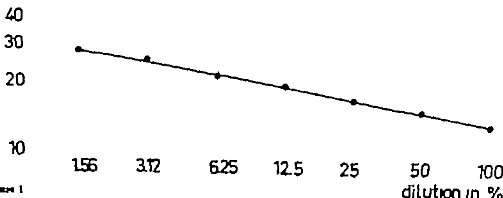


Figure 1

Relation between the Stypven-time and percentage of platelet factor 3. The clotting time of four times frozen and thawed platelet-rich plasma was taken as 100 % PF 3 (= total PF 3). The curve was constructed by estimating the clotting times of this plasma diluted with various amounts of platelet-poor plasma of the same individual.

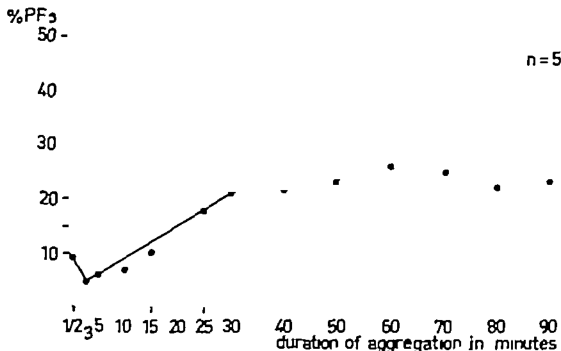


Figure 2

The percentage of PF 3 was determined in five separate experiments with 10 μM ADP at 37 C. The PF 3 availability is linear with time to 30 minutes after ADP-addition. The initial increase in PF 3-availability is clearly shown.

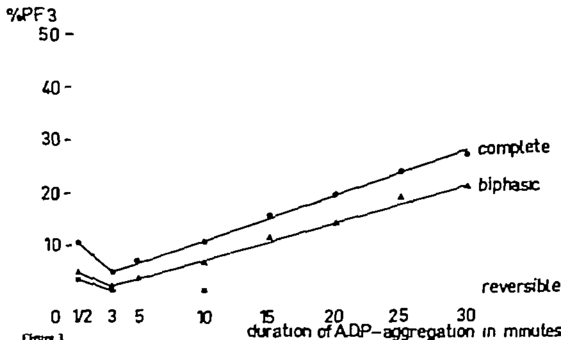


Figure 3

The PF3-availability in complete or bipha ADP aggregation does ot differ significantly. The availability in reversible aggregation is low

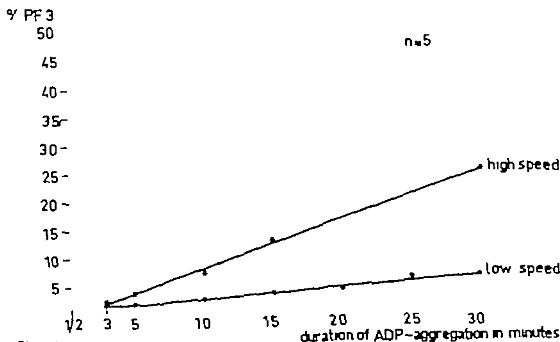


Figure 4

PF3 release t 37°C and t 10 µM ADP was st died t 750 rpm stirring rate and t 1150 stirring rate (pyromag-stir) The differences were significant in the ranked sign test ($P < 0.05$) (Courtesy of F K. Schattauer Verlag, Editors of Thrombos. Diathes. haemorrh.).

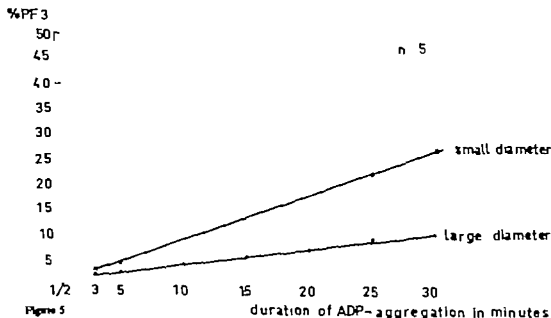


Figure 5

PF3 release t 37 C, 10 µM ADP and 1150 rpm were studied in ound bottom cuvette (contact area 790 mm²) and in flat bottom cuvette (internal diameter 13 mm, contact rea 620 mm) The differences ere significant in th ranked sign test ($P < 0.05$) (Courtesy of F K. Schattauer Verlag, Editors of Thrombos. Diathes. haemorrh)

convert clotting times into percentages, taking undiluted four times frozen and thawed platelet-rich plasma as 100 %. In this way we could demonstrate (1) that PF 3 is becoming available rather slowly during ADP aggregation. The curve starts with a slight amount of PF 3 available after 30 seconds. This amount decreases after 2 minutes, possibly by hiding of the changed membrane inside the aggregate which is subsequently formed. From then onwards platelet factor 3 is made available in a linear relation with time (Figure 2). We only found a slight amount of platelet factor 3 in reversible aggregation (Figure 3). The time sequence nor the temperature dependence of PF 3 – availability is in any way related to the release reaction as exemplified by the release of ^{14}C-labelled serotonin. Serotonin is released after 30 seconds reaching a maximum after 2 1/2 minutes and is absent below 33°C. PF 3-availability is present at 20°C and is linearly related with time till 30 minutes after the start of the aggregation at 37°C and till 90 minutes at 20°C. We found some indications that the amount of PF 3 available was related to mechanical trauma. Suggestive for this was our observation that stirring speed as well as surface area of the cuvette influenced the amount available (Figure 4 and 5).

This would mean that the availability of PF 3 in vitro during ADP-aggregation might in some way be an artifact of the in vitro situation. It is improbable that a similar mechanical trauma plays a role in the in vivo situation. This might mean that the initial small increase of PF 3 in the first few seconds is more important than is apparent from its size.

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15 PLATELET FACTOR 3 AVAILABILITY AND KINETICS OF PLATELET AGGREGATION

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During aggregation by aggregating agents such as ADP adrenaline and collagen platelet factor 3 is made available This phenomenon is now well known. However several questions are still unanswered

1 Whether factor 3 availability is a phenomenon depending on aggregation.
2. Whether factor 3 activity is increased by the release reaction during aggregation
3 Whether ADP is required to mediate the effect of other agents such as adrenaline and collagen on factor 3 availability

To investigate this problem, we have measured the shortening of Stypven time during aggregation of platelet-rich plasma (PRP) In each experience PRP contains the same number of platelets per cubic millimeter In our experimental conditions, stirring of PRP alone without any aggregating agent shortens slightly the Stypven time Therefore our results will be corrected for this slight artificial shortening. During some experiments, the release of nucleotides has been measured by the technique of firefly luminescence and the relase of 5-hydroxytryptamine (5 HT) has been measured after incubation of PRP with [1] C 5 HT With ADP 5 μM aggregation and shortening of Stypven time are simultaneous (Figure 1) At the concentration of 1,8 μM ADP induces a two-phase aggregation a shortening of Stypven time is observed as soon as the aggregation starts and the rate of this phenomenon does not seem to be modified when ADP and 5 HT are released (Figure) In two thrombasthenic subjects, ADP 5 μM does not induce aggregation whereas the Stypven time is shortened, even if to a less extent than in normal subjects (Figures 3 and 4). In our experimental conditions the release of ADP and 5 HT is diminished in both cases but is not absent Thus, it seems that there is no correlation between the degree of ADP induced aggregation and factor 3 availability neither in normal nor in thrombasthenic subjects

The kinetics of aggregation are well dissociated from those of factor 3 availability during the two-step aggregation induced in some PRP by a critical concentration of adrenaline (Figure 5) In this case a concentration of 0.5 μM of adrenaline is used during the slow progressive first phase factor 3 availability increases and reaches rapidly its early maximal values. Factor 3 availability is very important while there is no release yet (Figure 5). Aggregation induced by a purified collagen is accompanied by a progressive increase in factor 3 availability which already appears during the lag-phase (Figure 6) During this lag-phase no

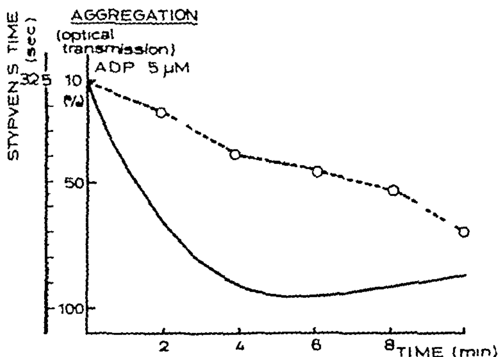


Figure 1

Aggregation of platelets ( ) and shortening of Stypven time (—) induced by ADP 5 μM added to PRP f a normal subject.

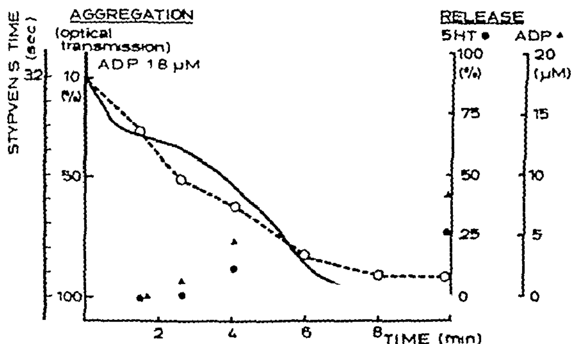


Figure 2

Aggregation of platelets ( ) shortening of Stypven time (—), release of 5 HT (●) and of ADP (▲) induced by ADP 1,8 μM added to PRP of normal subject.

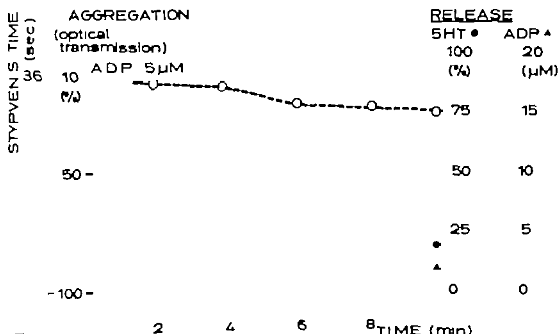


Figure 3

Aggregation of platelets (–) shortening of Stypven time (– –) release of 5 HT (●) and of ADP (▲) induced by ADP 5 µM added t PRP f thrombasthenic subject.

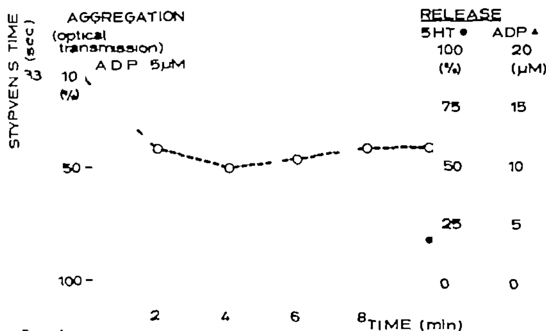


Figure 4

Same experiment as in figure 3 in another thrombasthenic subject.

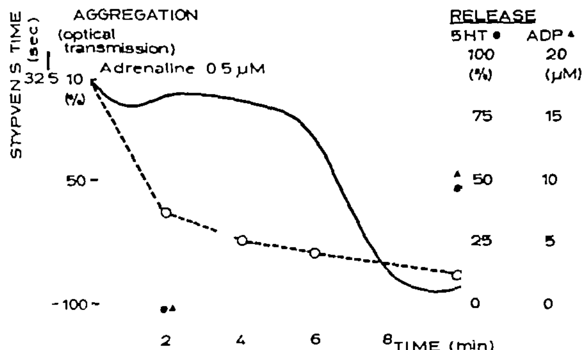


Figure 5

Aggregation f platelet ( ) shortening f Stypven time (----) release f 5 HT (●) and of ADP (▲) induced by adrenaline 0,5 µM added to PRP f a normal subject

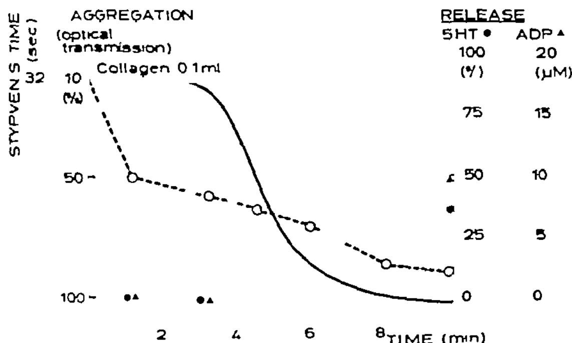


Figure 6

Aggregation f pla let ( ) shortening f Stypven time (----) release of 5 HT (●) and of ADP (▲) induced by suspension of purified collagen added to PRP of normal subject

aggregates are visible in the PRP at phase microscopy Moreover there is no release at this moment. These results seem to show that both adrenaline and collagen can induce an early modification of platelets which makes factor 3 available sometimes before or without appearance of aggregates and often before the release of ADP and 5 HT Moreover the rate of shortening of Stypven time does not seem significantly increased during or after the re lease reaction.

16 DISSOCIATION BETWEEN PLATELET FACTOR 3 AVAILABILITY AND PLATELET AGGREGATION*

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Platelet factor 3 (PF3) is made available during platelet aggregation induced by different agents. This phenomenon has been described by several authors (4 9 13) and confirmed in our laboratory using adenosine 5'-diphosphate (ADP) adrenaline and bovine fibrinogen. Weiss (19) has suggested that the release of ADP from the platelets is an essential step in the development of available PF3 This hypothesis is supported by the results of Hardisty and Hutton (8) who found that adenosine and 2-chloroadenosine substances which inhibit platelet aggregation by ADP also inhibit development of PF3 activity by incubation with kaolin. However Weiss (19) underlines that ADP even at high concentrations, is less potent than kaolin in making PF3 available this should indicate that the effect of kaolin is not entirely mediated by ADP Sixma and Nijssen (15) Horowitz and Papayoanou (10) and Atac et al. (1) have recently stressed the complexity of the relationship between PF3 availability platelet aggregation and release reaction

In this paper some data are presented which indicate that the results of platelet aggregation measured photometrically and of various assays for PF 3 availability do not invariably run in parallel. All the experiments have been performed at 37°C. Simultaneous recording of platelet aggregation and assay of available PF3 were performed as described by Hardisty and Hutton (9) platelet-rich plasma (PRP) was placed in a cuvette of the aggregometer the eventual inhibitors and aggregating agents were added subsequently occurrence of platelet aggregation was followed with Born's photometric technique (3) using an E.E.L. 401 Absorptiometer (Evans Electroselenium Ltd.) with built-in stirrer heat-exchanger and water jacket for temperature control. The absorptiometer was connected to a pen recorder (Vitatron) for automatic registration of the variations in transmitted light. At intervals, 0.1 ml of the mixture in the aggregometer was transferred to a tube in the 37°C waterbath containing 0.1 ml of 0.05 M $CaCl_2$ 0.1 ml of Russell's viper venom (Stypven) diluted 1/100.000 and stored at 4°C was then pipetted rapidly and the clotting time determined. Up to

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10 samples could be removed from the incubating mixture without interfering with the optical density recordings, since the initial level of fluid in the cuvette was well above the light path

1 We have recently described (7) a patient with severe chronic idiopathic thrombocytopenic purpura whose serum, after repeated transfusions of ABO compatible platelet rich plasma was found to contain immunoglobulin G capable of aggregating human platelets after a latent period and of making PF3 available in both stirred and unstirred PRP As shown in Figure 1 the shortening of Stypven time reaches its maximum during the latent period, i.e. before platelet clumping (detectable in the aggregometer) occurs.

2. The same phenomenon, even if less evident, has been observed in stirred PRP to which collagen (f.c. 40 μg/ml) was added as shown in Figure 2 the Stypven time becomes shorter already during the lag phase preceding the onset of aggregation.

When unstirred PRP was incubated with collagen (f.c. 40 μg/ml) neither platelet aggregation was observed in the aggregometer nor did the Stypven time become shorter however when a higher concentration of collagen (f.c. 0.4 mg/ml) was used the optical density did not change but the Stypven time was shortened as indicated in Table I

3 Both adenosine (f.c. $5\,10^{-4}$ M) and acetylsalicylic acid (f.c. 2.10^{-4} M) inhibit immunoglobulin-induced platelet aggregation but have no effect on the shortening of Stypven time (Figure 1).

4 Preincubation of PRP from normal subjects with acetylsalicylic acid or indomethacin (f.c. 2.10^{-4} M) does not modify the shortening of Stypven time induced by collagen (f.c. 40 μg/ml). Figure 3 shows the results of the simultaneous recording of aggregation and assay of available PF3 after addition of collagen to normal PRP in presence of indomethacin. It can be seen that to a strong reduction of aggregation does not correspond a significant modification of PF3 availability

5 Platelets preincubated at room temperature during 60 minutes with a combination of inhibitors of glycolysis (2-deoxy-D-glucose) or of oxidative phosphorylation (antimycin A) lose their ability to retract a clot (6,11) and to be aggregated by ADP (7 12) but make normally available immunoglublin-induced PF3 ac

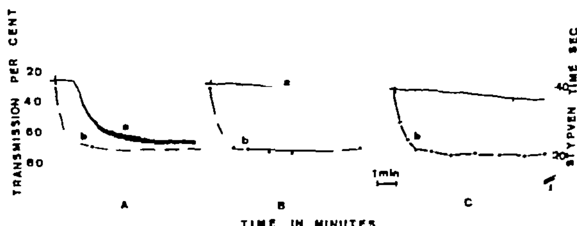


Figure 1

Simultaneous recording of platelet aggregation ( ) and assay of available platelet factor 3 (Stypven time) (b) after addition of 0.1 ml serum from patient with idiopathic thrombocytopenic purpura (see text) to
A 1.8 ml normal PRP + 0.1 ml isotonic saline
B 1.8 ml normal PRP + 0.1 adenosine (f.c. $5\,10^{-4}$ M)
C 1.8 ml normal PRP + 0.1 acetylsalicylic acid (f.c. 2.10^{-4} M)

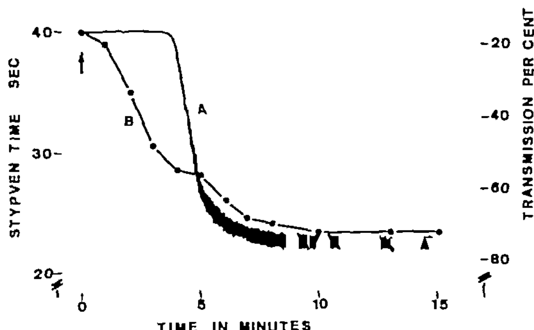


Figure 2

Simultaneous recording of platelet aggregation (A) and assay of available platelet factor 3 (Stypven time) (B) after addition of 0.4 ml calf-skin collagen Stago (f.c. 40 µg/ml) to 1.6 ml PRP (300,000 platelets/µl).

Table I

Effect of incubation of unstirred normal PRP (300,000 platelets/µl) with calf-skin collagen Stago (0.4 mg/ml f.c.) on Stypven time (seconds)

Time of incubation	Collagen	Control (0.1 N acetic acid)
0'	40.6	39.8
15'	28.2	38.6
30'	25.4	38.0

tivity (Table II). In contrast, PF3 development during ADP-induced aggregation was inhibited as well as aggregation.

PF 3 availability was also studied by the methods of Spaet and Cintron (16) and Weiss (19). Since in both methods kaolin is used as activating agent, it was not possible to obtain simultaneous recordings of platelet aggregation measured as changes in optical density. The alteration of PF3 induced by kaolin was thus compared to platelet aggregation provoked in other samples of the same PRP by different aggregating substances. The results obtained can be summarised as follows: kaolin-induced PF3 availability was not modified by a concentration of acetylsalicylic acid or indomethacin such that it was able to strongly reduce platelet aggregation by collagen (f.c. 40 µg/ml) or to inhibit the second wave of aggregation brought about by adrenaline (f.c. 4 µg/ml). In addition, we have not been able to find a significant inhibition of PF3 availability neither in 8 normal children after ingestion of 30 mg/kg body weight of acetylsalicylic acid (5) nor in 10 normal adults receiving 0.5-1.0 mg/kg body weight of indomethacin. Residual prothrombin in serum one hour after clotting was also normal in all these subjects at various intervals after ingestion of the drugs. However collagen- and adrenaline-induced platelet aggregation studied at the same time were inhibited.

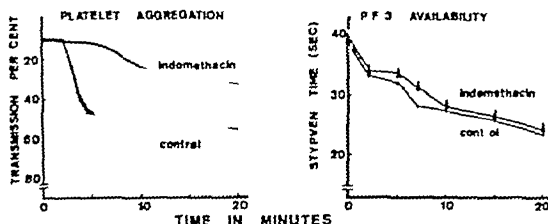


Figure 3

Simultaneous recording of aggregation and assay of available platelet fact r 3 (Stypven time) after addition of calf-skin collagen Stago (40 μg/ml f.c.) to stirred normal PRP (300,000 platelets/μl) Effect of 3 min. preincubation at 37 C with indomethacin (f.c. 2.10^{-4} M)

kaolin-induced PF3 activity was not modified by preincubation of PRP with a combination of metabolic inhibitors, as described above

The data presented here indicate that platelet aggregation measured photometrically in Born s aggregometer and the availability of PF3 are not invariably linked phenomena. This could be due to the test systems themselves f.i. it is known that some platelet aggregation can occur before a detectable decrease of optical density in Born s aggregometer is measurable this could partially explain some of our findings however PF3 was also made available after addition of immunoglobulin or collagen (at suitable concentrations) in a non-stirred system when no clumping occurred as confirmed by phase microscopy It is also possible that a variable amount of PF3 is made available in the test systems used in an aspecific way due f i to stirring, introduction of the pipette in the test tube for serial determinations of Stypven time lysis of a few platelets etc control experiments, however indicate that this aspecific activation of PF3 is not consistent These technical considerations, therefore do not explain our results The conclusion of some authors (1 10 15), that even f PF3 activation and platelet aggregation usually occur together they need not necessarily do so seems more likely

Our experiments performed using collagen or serum from a patient showing platelet clumping and PF3 activating ability suggest that both substances react on the platelet membrane inducing such a modification of the latter that PF3 is rapidly made available (unmasked) endogenous ADP is subsequently released leading to platelet aggregation if PRP is

Table II

Effect of metabolic inhibitors on PF3 availability induced by the serum of patient with idiopathic thrombocytopenic purpura (see text) Portions of 2 5 ml PRP were preincubated during one hour t room temperature with 0.06 ml 2-deoxy-D-glucose (Sigma) in isotonic saline (1M) and 8 μl antimycin A (Sigma) in ethanol 94 % / (1 mg/ml) Control PRP were preincubated with 0.06 ml isotonic saline and 8 μl ethanol. After preincubation, aliquots of 1 9 ml PRP were placed in the cuvettes of the aggregometer and 0 1 ml serum was added

Time after addition of the serum	Metabolic inhibitor	Control
	Stypven time (second)	
0'	35.2	35 0
5'	3 2	23 4
15'	2_8	23 0
30'	1 0	1 4

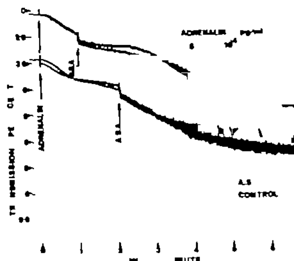


Figure 4

Inhibitory effect of acetylsalicylic acid (A.S.A.) on platelet aggregation induced by adrenaline. A.S.A. was added at two different intervals after adrenaline, during the first wave of aggregation elicited by this substance.

continuously stirred. Adenosine would not inhibit either the activation (exposure) of PF3 or the release of ADP but aggregation does not occur because of the competition of adenosine with the released ADP (2). Acetylsalicylic acid or indomethacin would not interfere with the primary reaction of platelets with these aggregating agents so that PF3 could be made available the release reaction however is inhibited (14 18,21) and aggregation does not occur The hypothesis that anti-inflammatory drugs do not interfere with the primary reaction between the platelet membrane and the aggregating agent is supported by the work of Spaet and Lejnieks (17) who found that the primary adhesion reaction between platelets and connective tissue fragments (collagen) is unaffected by acetylsalicylic acid. In addition, we have observed that both acetylsalicylic acid and indomethacin can reduce the aggregation induced by collagen or inhibit the second wave of aggregation elicited by adrenaline even if added after the aggregation inducer (Figure 4).

Our findings of a normal residual prothrombin activity in serum and of a normal PF3 availability induced by kaolin in subjects receiving acetylsalicylic acid or indomethacin are in agreement with the results of Weiss (20).

The findings that acetylsalicylic acid or indomethacin do not inhibit PF3 development by collagen cannot readily be explained by the observation reported by Atac et al. (1) that connective tissue particles themselves shorten the Stypven time indeed, at the concentrations used for inducing aggregation, collagen was not able to significantly shorten the Stypven time in unstirred PRP or in stirred platelet poor plasma this indicates that in our system the shortening of the Stypven time is almost entirely due to activation of PF3 and that anti-inflammatory drugs do not inhibit this activation

The results obtained in the presence of metabolic inhibitors indicate that the activation of PF3 by collagen, immunoglobulin or kaolin in contrast to the release reaction, is not an energy-dependent mechanism but mainly depends on the contact of these substances with platelets. The observation that metabolic inhibitors inhibit both aggregation and PF3 availability induced by ADP would suggest that PF3 activity developing during aggregation by ADP *is due to aggregation and not to ADP itself*

In conclusion the contact of platelets with an activating" substance seems to be the more important event for an optimal activation of PF3 It is not unlikely that a platelet should act as an "activating substance for another platelet, thus explaining the development or the increase of PF3 availability during platelet aggregation.

Acknowledgements

We wish to thank Miss Annie Vandenbussche for her skilful technical assistance.

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DISCUSSION

J.R O'BRIEN Professor Hardisty why did you choose imipramine in your first experiment, since it has effects on the platelets in the absence of ADP ? Secondly there appeared to be considerable aggregation. I wonder if release was occurring.

R.M HARDISTY I admit that imipramine was a badly chosen reagent because it can cause mechanical damage but the same phenomena can be demonstrated in the absence of imipramine by studying platelets which have disaggregated spontaneously after ADP aggregation, without release having occurred.

J.J SIXMA I would like to say that I absolutely agree with Dr Hardisty about his data on centrifugation. In studying the same problem in a slightly different way we found the same low levels of PF3 in the supernatant. We centrifuged ADP-aggregated platelet-rich plasma and studied at different time points the amount of PF3 in the supernatant, utilizing the described transformation of the Stypven times to percentages of PF3 We centrifuged at a low speed of 1000 g during 10 minutes but still only 20 per cent of PF3 was in the supernatant. More than 80 per cent is centrifuged down with the platelets. I completely disagree with Dr Hardisty on the amount of PF3 that is released or made available in reversible aggregation. There was a slight shortening of the Stypven time but when expressed as percentage of total platelet factor 3 we found only 3 per cent made available, never more (Figure 1)

R.M HARDISTY I think that the amount which is made available depends largely both on the degree of aggregation and the duration there is a lag between aggregation and the fall in Stypven time We did these experiments some years ago before anybody was looking for a second wave of aggregation or for evidence of the release reaction, and we used quite a high concentration of ADP which caused a marked fall of optical density before reversal. With a more transient primary aggregation and no second wave or release I would agree with you that little PF3 becomes available

J.J SIXMA The total amount of PF3 that is becoming available in reversible ADP-aggregation is present after about 30 seconds. Although it is negligible in terms of total PF3 or PF3 that is made available in due time in larger aggregates, it still may be of importance for the situation in vivo The large amount of PF3 that becomes available after prolonged stirring of rather large aggregates, might well be caused by the effect of mechanical trauma as our experiments on the rate of stirring and the importance of the surface area suggest.

S. CRONBERG When I was with Dr Caen in Paris, we did an interesting experiment on plasma from a patient with F XII deficiency We harvested native PRP without any addition of anticoagulant and aggregated with ADP at the time when a second wave could be expected, it came and was followed immediately by the coagulation of the plasma. If the same amount of ADP was used to aggregate the platelets but at the height of the aggregation prostaglandin was added which induced

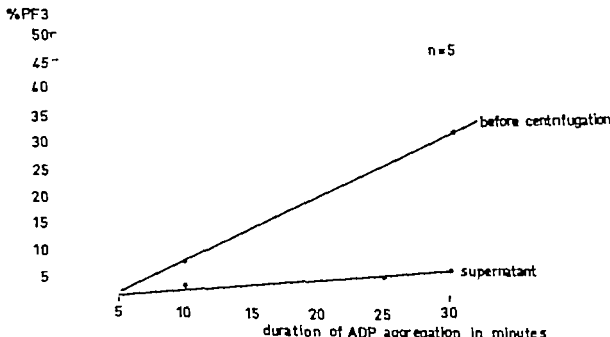


Figure 1

The amount of available PF3 was measured in the PRP subjected to ADP aggregation and in the supernatant after a short centrifugation at 1000 g. About 20 % of the available PF3 remained in the supernatant after centrifugation.

immediate disaggregation, then there was no coagulation for at least 10 min this suggests thrombin was more easily formed inside these large aggregates.

J.J. SIXMA I would like to comment on Dr Cronberg's observations we have similar findings in a different situation We studied the electron microscopic picture of ADP-induced aggregates in hirudin PRP We used high (150 u/ml) and low (25 μ/ml) concentrations of hirudin Both plasma's were unclottable When aggregates were studied that were fixed 10 minutes after the addition of ADP we observed fine fibrin fibers at the periphery of the aggregate This was present in the plasma with the low hirudin concentration not in that with the higher concentration I feel that this might be a system to study the relative importance of the intrinsic and the extrinsic system for thrombin formation during aggregation.

J.W. ten CATE May this finding be explained by paracoagulation of fibrinogen by release of platelet factor 4 ?

J.J. SIXMA I am not sure about that a clot formed by paracoagulation has just the same structure as fibrin but I think when you talk about paracoagulation, you always say that you have got fibrin monomers and that of course reflects development of thrombin too We have here a situation where you can study the development of thrombin after aggregation

A.A. SHARP I just want to recall another factor that can promote platelet factors release without propinquity and this is the mere fact of chilling plasma in the process of making native PRP If one does this, one finds PF3 release before aggregation occurs. It is also interesting to recall experiments I did 10 years ago when I had an artifactual system which produced rapid platelet aggregation. However one situation where the technique did not work was in platelet-rich plasma from patients with Hage-

man and Factor XI deficiency where platelet aggregation was very slow Therefore I think it would be interesting to consider in relation to PF3 release whether physical injury to platelets, or Hageman factor (Factor XII) and perhaps PTA (Factor IX) are necessary for this release mechanism.

R.M. HARDISTY I have a comment on Dr David's presentation. I do not think that the shortening of Stypven time is related quantitatively to the amount of available platelet factor 3 This is illustrated in Figure 2 which shows the Stypven times of serial dilutions of platelet-rich plasma in platelet-poor plasma from the same subject there is in fact a linear relationship between the Stypven time and the logarithm of the platelet count, whether the platelet lipid has been made available by aggregation with ADP (upper curve) or by freezing and thawing (lower curve) It is from dilution curves such as these that I have inferred that about 20 per cent of the total phospholipid clotting activity of platelets is made available by maximal aggregation The Stypven time of intact PRP in these experiments was about 30-40 sec. so that the percentage shortening after aggregation or freezing and thawing is much greater in proportion to the platelet count at low concentrations of PRP than at high ones. This seems to me to show that it cannot be used for the quantitative measurement of available PF3 since the availability of very little PF3 will result in proportionately much greater shortening of the Stypven time

J CAEN May I ask Dr Hardisty at which temperature he has done these various centrifugations, and may I ask if he has some differences in the availability of PF3 depending on the temperature you use

R.M. HARDISTY The actual centrifugation

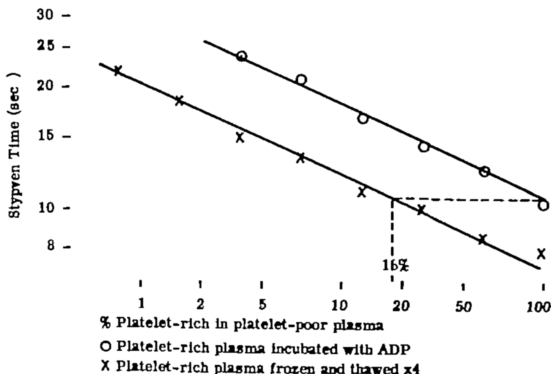


Figure 2

Relation of Stypven time to platelet count.

we have done at room temperature perhaps a little bit above but not in the cold. I would agree with Sharp s comment on the effect of cold on PF3 availability

J CAEN Does anybody know how the anti-inflammatory agents possibly act on PF3 ?

R.M HARDISTY Not as far as I know I have not used indomethacin at all but I cannot agree with Dr de Gaetano on the lack of effect of aspirin on PF3 availability We have not studied this systematically but in a variety of patients with defective release whom we have studied whether this was brought about by aspirin or in other ways (e.g. in albinism) we have observed a defect of PF3 availability though a much less severe one than in thrombasthenia.

J.R. O BRIEN I would like to support Hardisty If release is inhibited there will be inhibition of PF3

P.M MANNUCCI I would simply like to remind that with our inhibitor BBA that apparently seems to act on the release reaction, we found a close correlation between the inhibition of the second wave of aggregation induced by ADP and that of PF3 availability using a kaolin system.

J.L. DAVID Adrenaline at 5 micromolar concentration induces aggregation with shortening of Stypven time Preincubation of PRP with imidazol inhibits completely aggregation of platelets by adrenaline with small inhibition of PF3 availabilty Release of ADP and release of 5HT are normal without or with imidazol there is therefore a very complete dissociation between aggregation and PF3 availability

J.J SIXMA I would like to ask Dr Hardisty or anybody present, if he has any idea on the localisation of available PF3 in an aggregate Do you suppose that the membranes on the outside of the aggregate have changed or can it arise somewhere inside the aggregate ?

R.M HARDISTY I think that is just any body's guess I do not see why it should not be in the slits between the adjacent platelets, though presumably that on the outside would have the most obvious effect

S CRONBERG I think that the conditions inside the large aggregates might be quite different from the conditions in the plasma Probably metabolic reactions take place inside the aggregates that do not occur among the freely dispersed platelets in the plasma Thus citrate might be metabolized and reactions take place that promote thrombin formation inside the aggregates (1)

J.J SIXMA The ultrastructure of the aggregates formed in plasma with low hirudin concentration on which I commented earlier in this discussion showed fibrin in the periphery of the aggregates but there is some fibrin inside the aggregate too The situation is different however when we measure the presence of PF3 with a Stypven time It is most probable that factor X which is activated with Russell's viper venom will adsorb to the membranes on the outer side of the aggregate and will act there Diffusion of this activated factor in the short time of the Stypven time determination seems less probable to me although I admit that I might be wrong.

J.R. O'BRIEN We have several times heard of differences between ADP-induced aggregation and that induced by adrenaline There is no shape change with adrenaline There also seems to be no heparin neutralising activity or PF4 on the surface heparin neutralising activity is however released later but only if the normal release reaction occurs.

J CAEN We have published 4 or 5 years ago some results on thrombasthenic platelets showing an abnormal aggregation and an abnormal platelet factor 3 availability as expressed by the Stypven time

J R. O BRIEN A question to Dr Holmsen He

said that in his release studies platelet factor 4 was released and mucopolysaccharides were released. Would he please tell me if he thinks these were in any way related events.

H HOLMSEN Unfortunately I cannot tell you anything about it because we have not done any kinetic studies to determine if they are released in parallel. Neither do we know to which extent the mucopolysaccharides are located in the granules.

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Question N 6

DOES RAPID DISAGGREGATION FOLLOWING ADP INDUCED AGGREGATION HAVE ANY SIGNIFICANCE ?

DISCUSSION

R.M HARDISTY
G de GAETANO
J.R. O BRIEN
G V R. BORN
E.F LUSCHER

H. HOLMSEN
F MICHAL
J.J SIXMA
J CAEN

17 PLATELET DISAGGREGATION AND REFRACTORINESS TO ADP

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In 1968 we published some experiments (2) on *a platelet property named the refractoriness of platelets* when platelet-rich plasma (PRP) and ADP are gently mixed and allowed to stand for a while without stirring, there develops a very strong inhibition of the aggregation caused by a second ADP addition with stirring. This is shown in Figure 1 where 15 μM radioactive ADP have been used to induce refractoriness Already after five minutes there is 90 % inhibition of platelet aggregation, induced by a new addition of 1 μM ADP and stirring in an aggregometer This inhibition persists over a period of two hours and does not at all correlate with the breakdown of the radioactive ADP in plasma In the upper figure C^{1} ADP and PRP are incubated without added adenosine deaminase and the adenosine produced could theoretically be the cause of the inhibition observed In the lower figure adenosine deaminase is present in PRP and no adenosine is developing Still there is marked inhibition, which consequently can not be due to adenosine Table I shows that this peculiar sort of inhibition is dependent on the amount of ADP we use fo inducing aggregation and on the

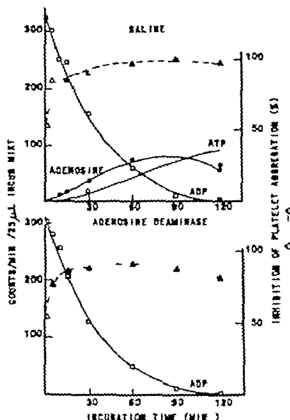


Figure 1

Metabolism of C^{1} ADP at 37 C in PRP (3,3 x 10^{5} platelets /µl) and determination of inhibition of aggregation (induced by 1 µM ADP) in the absence and presence of adenosine deaminase For details see reference 2.

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Table I

Effect f increasing concentrations of (aggregating) ADP on inhibition of platelet aggregation by ADP

PRP was incubated with ADP (15 µM) at 37 C for 60-90 minutes and the rate of aggregation was measured with different concentrations of ADP

Conc. of (aggr.) ADP (µM)	0.64	1.6	3.2	8.0	30.0
Per cent inhibition	81	70	57	31	16

amount of ADP we use to induce inhibition varying the concentration of "aggregatory" ADP (second ADP addition with stirring) and keeping the first added ADP at a constant level, inhibition becomes less as the concentration of aggregatory ADP is increased. Figure 2 shows that the inhibition also is a property caused by ATP With ATP the contribution of adenosine induced inhibition is greater However it is not certain whether the effect of ATP is directly on the platelets or *via* formation of ADP

Figure 3 shows aggregometer tracings of platelet aggregation induced by 0,5 µM C^{14} – ADP with simultaneous determination of ADP-metabolite radioactivity The platelets (*left tracing*) aggregate and disaggregate while ADP is present in PRP and *slowly* broken down small amounts of adenosine formed. In the right tracing there is the same PRP with the same amounts of ADP but now large amounts of adenosine deaminase are present In this system no adenosine is formed, but neither the degree of disaggregation nor ADP breakdown were altered. Platelets had disaggregated 50% the ADP amount was only degraded 10% Apparently there is a lack of

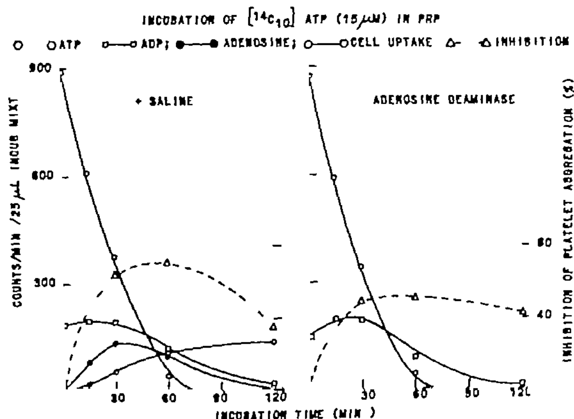


Figure 2

Variation in the radioactivity of ATP metabolites during incubation f ATP t 37 C in PRP (2,9 10^{5} platelets /µl) and determination of the inhibition of the aggregation induced with 1 µM ADP in absence and presence of adenosine deaminase Further details are given lsewhere (2)

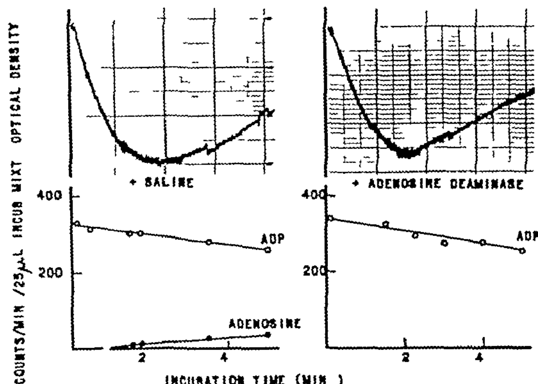


Figure 3

Simultaneous study of aggregation-disaggregation of platelets induced by 0,4 μM C ADP to PRP (3 8 10^5 platelets /μl) at 25 C and metabolism of C ADP in the absence and presence of adenosine deaminase

correlation between disaggregation and ADP breakdown.

When ADP and PRP were stirred together (Figure 4) aggregation and disaggregation occurred as described above The tracing of an aggregation which was interrupted by the addition of potato apyrase is shown in the middle (Figure 4) Immediately after addition of this ADP-removing enzyme the platelets disaggregated rapidly and now it was good correlation between disaggregation and the disappearance of ADP The very right column of curves (*Figure 4*) *illustrates a situation when apyrase was added in the middle of aggregation in the presence of adenosine deaminase Still we have rapid disaggregation caused by apyrase and this phenomenon could not be due to adenosine* development.

Summarizing, we have shown that the presence of ADP is an absolute requirement for aggregation, if ADP is removed very rapidly during aggregation, a very rapid disaggregation occurs which is not due to adenosine formation. On the other hand when the platelets aggregate and disaggregate by themselves, there is a very peculiar lack of correlation between disaggregation and the disappearence of ADP More likely disaggregation is caused by the above described property refractoriness of the platelets towards ADP Our results have been verified by Packham et al. (1).

The platelet refractory period might have three serious technical implications First, in electrophoretic studies of platelet mobility platelets are mixed gently with ADP put into a chamber and an electric field is applied As they are not stirred in this chamber they are just in the conditions we saw in Figure 1 where

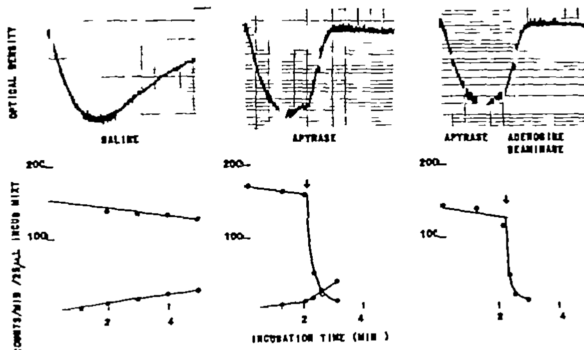


Figure 4

Simultaneous study f aggregation-disaggregation of platelet induced by 0,2 µM C^{14} ADP in PRP (3,9 10^5 platelets /µl) at 25°C and the metabolism of C^{14} ADP Effect f apyrase with and without adenosine deaminase

the refractory period was developed So most electrophoretic studies might actually have been done on platelets which have no ability to aggregate at all because they are refractory Second, ADP-induced platelet aggregation has often been used to measure ADP *breakdown*. This is usually done by mixing ADP with 2 samples of PRP by aggregating one sample immediately and the other after a variable period of incubation at 37°C the difference in the aggregating response has been taken as a measure for ADP breakdown We think that this measures *both* the refractoriness which has been developed, and breakdown of ADP The third implication is perhaps the most serious. When we prepare a sample of blood, it is impossible at least with current techniques, to avoid a small degree of haemolysis, by which ADP is set free to the plasma from red cells. This ADP will make the platelets refractory we feel this has been paid little attention to The amount of ADP which might leak off erythrocytes and even from platelets if the release reaction is induced, might vary so much from sample to sample even from the same person that it indeed might explain the irreproducibility in the findings of platelet aggregation.

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18 PLATELET DISAGGREGATION AND THE DEGRADATION OF ADP IN THE PLASMA

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For Holmsen and Rozenberg (see contribution n 17 of this Conference) platelet disaggregation in normal controls is not related to the rate of ADP breakdown by normal plasma due to the fact that they find disaggregation at a time when ADP is still present in the plasma at a dose able to promote platelet aggregation In some particular cases we have been able to observe that an increase of ADP breakdown by the plasma was related with earlier platelet disaggregation. This is the case of platelet behaviour of some mammalians (5) in chronic myelocytic leukemia (6) in children and in some human populations (1)

Another factor also to be considered accurately fo the disaggregation velocity is the inhibitory effect of adenosine on platelet aggregation increased adenosine incorporation into platelets could partially explain increased platelet disaggregation

We have previously been able to demonstrate (5) that platelet disaggregation is increased in rat platelet-rich plasma (PRP) and that ADP breakdown by rat plasma is 20 times as fast as that of human plasma (Figure 1)

In guinea-pig, ADP-induced platelet aggregation is stronger than with human platelets, and the disaggregation is reduced. We have shown (2) that C -adenosine was not incorporated in guinea-pig platelets as it is in human, dog and rabbit platelets. The platelet ability to incorporate adenosine could therfore be an important factor in the platelet disaggregation rate (Figure).

In chronic myelocytic leukemia, when ADP induced platelet aggregation is performed in leukemic PRP aggregation is often reduced and disaggregation increased (6) but the washed platelets behaviour is always normal (3). The leukemic plasma is able to breakdown ADP several times as fast as normal plasma In the platelet poor plasma (PPP) of these patients, adenosine deaminase activity is increased (4). The same plasma inhibits C^{14}-adenosine incorporation in leukemic platelets (4) as well as in normal platelets. This could be related to increased white cells breakdown and increased ADP and enzymes release ADP release in the plasma could be responsible for a refractory state of platelets to a new ADP stimulation and cell enzymes release could be responsible for increased ADP breakdown.

We were able to perform an interesting observation about the platelet behaviour in people living at an altitude over 12,000 feet in Peru and Bolivia (1) These populations had increased hematocrit and high fibrinogen level although they had very rare thrombotic disorders. The ADP concentration used to induce platelet aggregation in normal European people

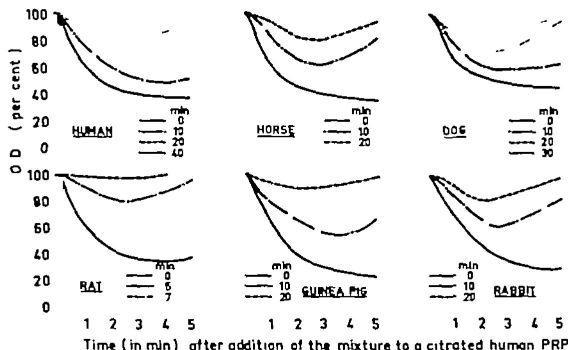


Figure 1

Aggregating activity f the mixture of ADP (5 10^{-6} M) and PPP of various species, incubated t 37 C and estimated photometrically on human citrated PRP

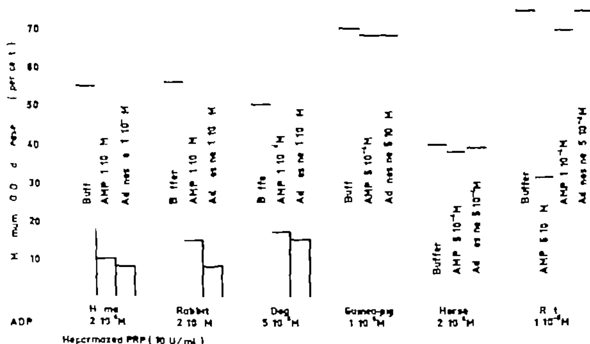


Figure 2

Effect of preincubation 5 min t 37 C f AMP and adenosine with citrated mammalian PRP on platelet aggregation in presence f ADP

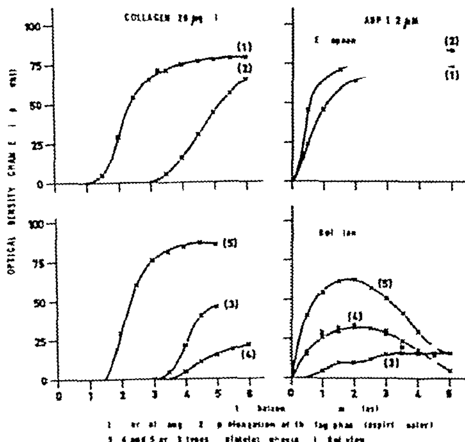


Figure 3

Collagen- and ADP-induced platelet aggregation in Europeans or Bolivians.

gave a rapid disaggregation (1) in Bolivian people. Breakdown of ADP by plasma is sometimes increased in these subjects. Collagen-induced platelet aggregation in Bolivians looks like collagen-induced aggregation after aspirin ingestion (Figure 3). These findings in normal subjects must be correlated with the observations we made using children platelets. ADP platelet aggregation is normal, disaggregation begins earlier and ADP breakdown is increased in the plasma.

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DISCUSSION

R.M. HARDISTY As regards human platelets, rapid disaggregation following ADP-induced aggregation simply reflects a lack of the second phase of aggregation. In various other species such as the rat one gets regularly a very rapid disaggregation because of a high rate of degradation of ADP in plasma but as far as I know nobody has claimed or has serious evidence that this is a relevant mechanism in man in other words those people in whom one can demonstrate a rapid disaggregation in association with a bleeding tendency fall into the group who have a failure of the release reaction none of these as far as I know have been shown to be due to an increase in the rate of ADP breakdown.

G. de GAETANO As Dr Hardisty has pointed out disaggregation following ADP-induced aggregation is present when the release reaction does not occur since anti-inflammatory agents do inhibit the release reaction it is likely that ADP-induced aggregation should be reversed by these drugs. We have indeed observed the presence of a rapid disaggregation in 12 out of the 16 patients examined this year whose platelet dysfunction could be surely related to ingestion of acetylsalicylic acid or indomethacin. In vitro experiments have confirmed the results obtained in vivo. Indeed, preincubation of PRP with acetylsalicylic acid or indomethacin induces or enhances the disaggregation after addition of ADP. The degradation of ADP studied as described by O'Brien (3), was found to be unmodified in the presence of either acetylsalicylic acid and indomethacin therefore an enhanced ADP breakdown by these drugs could be excluded. As a result we think that the reversal of ADP-induced aggregation in presence of anti-inflammatory drugs is the equivalent of the inhibition of the second wave.

G.V.R. BORN The results presented by Holmsen are important and his comments seem to me to be right and justified. We have been worried about electrophoresis observations. Haemolysis with release of ADP is likely to account for the fact mentioned previously that aggregability tested when plasma is first prepared is less than after it has stood around and any ADP has disappeared.

J.R. O'BRIEN I agree with much of what Dr Holmsen says. His sophisticated techniques agree with some old simple observations (2). However we have one piece of evidence in vivo which argues against the in vitro concept that platelets incubated in ADP or adrenaline become refractory to ADP or adrenaline. We studied platelet aggregation induced by adrenaline before and after violent exercise in some trained athletes in America. We must assume that the plasma catecholamine levels rose during exercise but the aggregation response to adrenaline after adjustment for the altered platelet count remained essentially the same.

E.F. LUSCHER Dr Holmsen has cast doubt on the results of electrophoresis experiments on the intact platelets. Nevertheless, these experiments which show that a short-lived increase in negativity is followed by the tendency to develop a positive charge seem quite con-

vincing for me this was up to now a very positive indication for a rapid change in charge distribution on the platelets surface

G V R. BORN It is not so short-lived it depends on the concentration

H. HOLMSEN It was theoretically I have started to doubt that electrophoresis does not measure stickiness but does measure refractoriness. I would never deny that charge changes are very likely to be involved to make platelets adhere to each other

G V R. BORN I have always been impressed by the artificiality of the system. There is a long time between the addition of the cells and of the agent Mills showed that ADP is broken down in plasma by two enzymes. One is myokinase or adenylatekinase which leaks from red cells (the activity of it is proportional to the amount of haemolysis) and which has very little activity on ADP at low concentrations. The other enzyme is an ADPase of which there is very little in the plasma but which does not depend on haemolysis and has a high affinity for ADP

H HOLMSEN Inger Holmsen has purified the ADP-ase of human plasma (Abstr F.E.B., 6th Congr., Madrid 1967 no 1045) This enzyme is very peculiar in its unspecificity It degrades CDP GDP IDP and UDP at the same rate as ADP It also splits ATP and AMP and has transphosphorylating activity These are properties of serum alkaline phosphatase but the ADP-ase from human plasma has a different elution pattern on ion exchange chromatography than the phosphatase We wondered whether the ATP-ase AMP-ase and ADP-ase of *plasma is one and the same enzyme* the relative activity of ATP-ase ADP-ase AMP-ase changes during purification which might mean they are three different enzymes furthermore in the condition of thyrotoxicosis we have three- or fourfold elevation of ADP ase whereas the ATP-ase level is normal This might also suggest that these are different enzymes

F MICHAL I do not know if the rapid disaggregation following ADP-induced aggregation has any significance mainly because I really do not know how to measure this phenomenon reliably *in vivo* My contribution to this question is to show that by the use of some substances we can influence the rate of disaggregation *in vivo* and *in vitro*. I am referring here to a specific ADP inhibitor 2-methylthioadenosine 5'-monophosphate (1)

Figure 1 shows an experiment in which 2-methylthio-AMP was injected into dogs at intervals we found that a small dose (350 micrograms per kg intravenously) given about every two hours reduced the responsiveness of platelets to ADP Samples of blood were taken at intervals before and after the injection of the nucleotide PRP was prepared and the responsiveness of platelets to ADP was expressed as percentage of responses obtained before the injections of the drug commenced. We also observed that platelet aggregates disaggregated more rapidly after the injection of 2-methylthio-AMP Disaggregation could also be speeded up in normal PRP by introducing 2-methylthio-AMP into PRP previously aggregated with ADP (but not with 5-HT) In figure 2 we have a demonstration that the inhibitory effect of 2-methylthio-AMP can be overcome by increasing the concentration of ADP but platelets pre-treated with 2-methylthio-AMP disaggregated rapidly whereas platelets not treated with the nucleotide aggregated with a smaller concentration of ADP irreversibly These experiments show that 2-methylthio-AMP injected either *in vivo* or *in vitro* causes some changes in the platelets which make them less responsive to ADP and allow them to disaggregate more rapidly The clinical significance of this observation is not clear but it would undoubtedly be useful to be able to cause platelets to be less responsive to aggregating stimuli and to disaggregate *in vivo*

J.J SIXMA We have observed that RA 233 and VK 744 two dipyridamole congeners, when added after ADP aggregation cause an active disaggregation this has no relation to

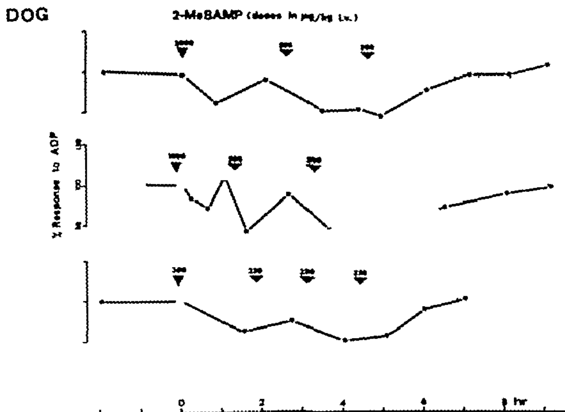


Figure 1

Effect of the intravenous administration of 2-methylthio-adenosine-5'-monophosphate to dogs on the response of platelets to ADP Points represent times of obtaining blood for the preparation of PRP Responsiveness to ADP is expressed as per cent of pre-treatment responses

the inhibitory effect of the drug on the first phase of ADP aggregation.

J CAEN I wish to ask Prof Hardisty whether he has found differences with regard to formation of second wave between adults and children do you use the same amount of ADP for both It seems to me that the degradation of ADP in the plasma of young children is more important than in normal adults

R M HARDISTY No, we have not noticed any difference between adults and children. It is not a subject I have systematically investigated from that point of view

G de GAETANO We have some preliminary results indicating that the concentrations of ADP adrenaline or collagen required to aggregate platelets obtained from cord-blood, are much higher than those we use in normal adults.

J R O BRIEN It seems possible to me that disaggregation occurs when there is an equilibrium across some membrane Perhaps there is aggregation when there is a high concentration of ADP outside the membrane and after 30 seconds there is an equilibrium reached and there is no further aggregation

Studying operation patients we get a very close correlation between the faster aggregation slope and the slower disaggregation slope when thrombin is the aggregating agent The

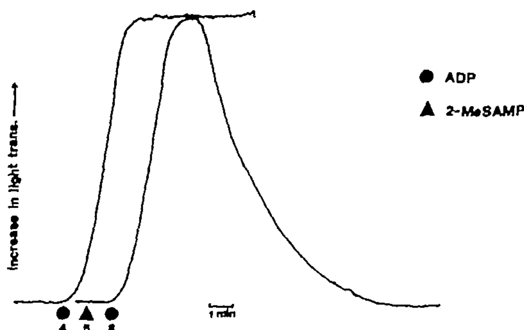


Figure 2

Platelet aggregation in dog PRP caused by ADP alone (first trace) and ADP in the presence of 2-methylthio-AMP (second trace).
Numbers represent final concentrations of agents in μM.
Temperature = 37 C

two phenomena must be very closely connected. With ADP a correlation of the two slopes exists but is less good this suggests that other factors may influence the speed of these reactions.

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Question N 7

ARE COMPARABLE RESULTS OBTAINED WITH DIFFERENT "COLLAGEN" PREPARATIONS ?

19 Y LEGRAND and G. PIGNAUD Some Factors Influencing the Aggregating Property of Collagen.

20. G BAELE, P VANDEN BOGAERT and F BARBIER Comparison of Platelet Aggregation in Normal Individuals and Uraemic Patients with Two Different Collagen Preparations.

DISCUSSION

J HUGUES
J R. O'BRIEN

J CAEN
H HOLMSEN

19 SOME FACTORS INFLUENCING THE AGGREGATING PROPERTY OF COLLAGEN

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Platelet aggregation induced by a purified collagen preparation can be quantitatively studied in a photometric test according to Born and Cross (?) at room, or better constant temperature in a thermostatized system (33°C or 37°C) The aggregation is measured by the optical density decrease of a platelet-rich plasma (PRP) put in the cuvette in contact with collagen. The appearence of the curves obtained is usually similar They can be divided into two parts a lag-phase very often characterized by a slight increase of the optical density then the optical density decrease corresponding to the aggregation phenomenon. With the same collagen preparations used in the same conditions in various experiments, the results can be reproducible (3). When different preparations are used, or when the conditions are changed the duration of the lag-phase the velocity of the aggregation and its intensity can vary Some factors can play a role in this topic.

1 *Animal Species and Organ (Starting Material)*

Any statistical study has been done about the relationship which could exist between the animal species or organ and the aggregating properties of the purified collagen extracted. But some differences have been observed (6) in the composition of collagen in different animal species. For instance some discrepancies are observed in the basic amino-acid content, and mainly in the lysine content of man or calf skin (27 residues per 1000) and rat skin (29 residues per 1000) and rat tendon (36 residues per 1000). According to the crucial role which after Wilner et al. (8) should be played by lysine in platelet adhesion to collagen, it could be possible that these discrepancies could involve some differences in the aggregation properties of collagen extracted from different species or organs.

2 *Age of the Animal*

We have previously shown (4) that the aggregating effects of an embryo-calf skin collagen was more important than the effect of a calf-skin collagen. For identical collagen concentrations (measured by the hydroxyproline content) In the same conditions of use the lag-phase was shorter and the optical density decrease more important with the younger collagen Differences were also observed in the release of aggregating activity from platelets after a five minutes contact with collagen at 33°C On another hand, slight diff

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also observed in the content in carbohydrates associated with collagen The same type of results was obtained by Bankowski et al (1) with crude extracts of connective tissue ex tracted from human skin or aorta at different ages hydroxyproline contents were always the same but aggregating activity decreased while carbohydrates content increased following the ageing process.

We think that the reduction of the aggre gating activity could be related to an increase in the number of the intra-molecular and inter molecular cross-links of collagen during the ageing process. Collagen cross-linkage involves particularly basic amino-acids positively char ged and mainly lysine and hydroxylysine. Other types of bonds such as hydrogen bonds in-volving hydroxyproline also appear Cross-linkage is a gradual phenomenon which explains some differences existing between an old and a young collagen chemical inertia, insolubility resistance to non-specific proteases, and also decrease of the aggregating activity The results obtained by Wilner et al. (8) show that the same amino-acids (lysine) are involved in both collagen cross-linkage and adhesivity It seems that the older collagen is, the less available the residues responsible for platelet adhesion are

3 *Purity of the Preparation*

Another very important point is the purity of the preparation It is absolutely impossible to compare two different collagens if they have *not been well purified. Collagen can particular* ly be contaminated by carbohydrates or derivatives such as hexosamines or uronic acids. A chemical analysis is necessary to know the content of these substances, though their exact role is not well established. But it seems that carbohydrates and derivatives have an inhibitory effect on the polymerization of collagen. This fact can be observed n vivo since in organs with a high carbohydrate content collagen fibers are thinner than in organs with a low carbohydrate content (for instance in cornea, which contains more carbohydrates than tendon collagen fibers are thinner). In vitro we have observed (4) that after addition of carbohydrate to a purified collagen solution the polymerization was markedly delaid. Two kinds of explanations can be given to this fact 1) the carbohydrates added should be fixed by the basic amino acid responsible for the poly merization (it is known that hexoses are located at the level of hydroxylysine as hydroxylysine-glucosyl-galactoside or hydroxylysine-glucoside units). 2) Carbohydrates could, after Grant et al. (7) separate the fibrils of collagen, in relation to the size of sugar molecules which are hydrated in these conditions. What is important on this point is that it is necessary to make sure that the preparation used is pure enough to discard any risk of interaction with non-identified contaminating substances, if they are present at a too important concentration. For our own part, the collagen we use is identified by physico-chemical methods infra-red and ultraviolet spectra hydroxyproline to nitrogen ratio amino acid analysis, hexoses, hexosamines, uronic acid content, these last two being the sign of a contamination by glycoproteins and acid mucopolysaccharides. The hydroxyproline to nitrogen ratio is most impor tant and has to be equal to 0.8.

A good standardization of the conditions of extraction and purification has to be realized to obtain this result In every aggregation test crude extracts of connective tissues have to be absolutely discarded since it is quite impossible to know the exact composition of the rea-gent" used.

4 *Standardization of the Conditions of Use*

The age of the animal and the contamination by carbohydrates seems to be related with the ability of collagen to polymerize into fibrils in vivo as in vitro The presence of fibrils seems to be crucial for the adhesion property of collagen. For instance urea impairs the poly merization of collagen, breaking the hydrophobic cross-links, and this phenomenon inhibits the adhesion of the platelets (5).

It is difficult to determine exactly the degree of polymerization in the photometric test. It is however possible to standardize the conditions of use of the collagen in order to have always

the same duration of the lag-phase for a control PRP (300 000 platelets/μl) since this duration seems to be directly linked to the amount of fibrils in the suspension The polymerization is obtained by heating the collagen solution at neutral pH at a temperature included between 25°C and 37°C. Collagen tends then to form a gel of fibrils. Our conditions are as follows incubation of a 200 μg/ml solution of collagen at 33°C during a time which has to be established for each sample (usually close to 7 min) This temperature seems to be the best one at 33°C polymerization is fast enough and the risk of an eventual thermic denaturation of collagen as could occur at 37°C is discarded. Polarimetric tests we recently did with guinea-pig and calf skin collagen showed us that at 37°C important amounts of collagen were denatured.

0 1 ml of this incubated collagen are put into contact with 0.9 ml of citrated PRP and the test is carried at 33°C. In these conditions, the lag-phase is usually equal to 1 min 30 sec, and the velocity of the aggregation which also depends on the PRP varies between 3 5 and 4 cm/min.

In conclusion platelet-collagen interactions must always be studied with the same collagen preparation (when platelet aggregation is tested) or in the presence of a control collagen obtained from the same animal species, at the same age in the same conditions of extraction, purification, and use (when collagen aggregating activity is tested)

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20 COMPARISON OF PLATELET AGGREGATION IN NORMAL INDIVIDUALS AND UREMIC PATIENTS WITH TWO DIFFERENT COLLAGEN PREPARATIONS

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The results of platelet aggregation with two different collagen preparations are compared in a group of 30 normal individuals and a group of 30 uremic patients.

As collagen preparations are used

1. Collagen Sigma 1 g is dissolved in 20 ml isotonic saline Maximal platelet aggregation is produced by adding 0.1 ml of this solution to 1 ml of platelet-rich plasma (PRP)
2. Collagen prepared from human tendons as described by Stuart (1) Maximal platelet aggregation is obtained when 30 μl of this preparation is added to 1 ml of PRP

Initially we intended to replace the collagen solution of Sigma by a cheap and easily made own collagen preparation.

The observation that the platelet aggregation with the Sigma collagen was grossly disturbed in an uremic patient with overt bleeding symptomatology in contrast to a subnormal aggregation with our own collagen preparation, was the reason to perform this study

Platelet aggregation is studied photometrically in a slightly modified EEL platelet aggregometer connected with a recorder A filter of 605 mμ is placed between the light source and the photoelectric cell. 0 % transmittance is adjusted by blocking the light beam with dark paper Platelet poor plasma (PPP) provides 100 % transmittance The transmittances are converted into optical densities.

Quantitative parameters used are (Figure 1)

- The reaction time (RT) expressed in mm (40 mm = 1 min) It begins at the moment collagen is added and ends at the beginning of the wave of aggregation
- $\frac{\Delta E}{\Delta t}$ the slope of the rapid aggregation
- ΔE_5 the difference in optical density between time 0 and 5 minutes.

Results

- The reaction time is markedly prolonged in uremic patients with both collagen preparations No correlation with the level of uremia is found. The reaction time is significantly shorter with the own collagen than with the collagen preparation of Sigma in both patient groups (Figure 2).
- The slope $\frac{\Delta E}{\Delta t}$ is significantly smaller in uremic patients. No difference is found between the two collagen preparations (Figure 3).
- ΔE_5 is smaller in uremic patients. In normal individuals a difference between the two collagen preparations is found (Figure 4). This may be related to the higher initial

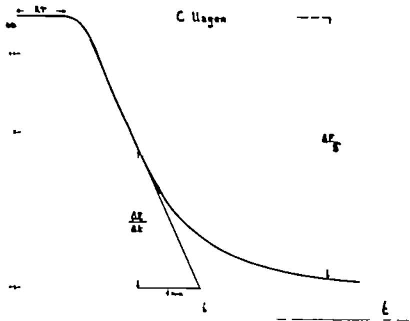


Figure 1
Quantitative parameters used.

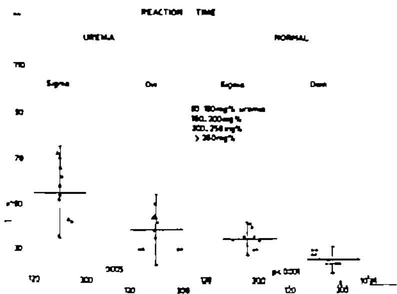


Figure 2
The reaction time w th th tw ollagen preparation in normal individuals and uremic patient

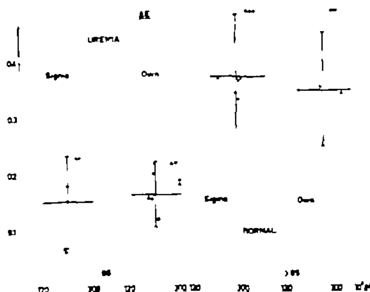


Figure 3

The slope of the rapid aggregation $\frac{\Delta E}{\Delta t}$ with the tw collagen preparations in normal individuals and uremic patients.

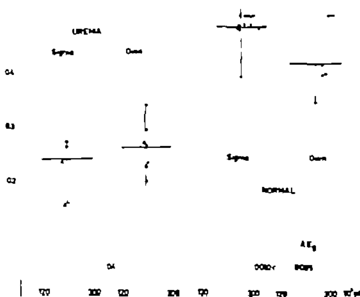


Figure 4

The difference in optical density between time 0 and 5 minutes (ΔE_5) with the two collagen preparations in normal individuals and uremic patients.

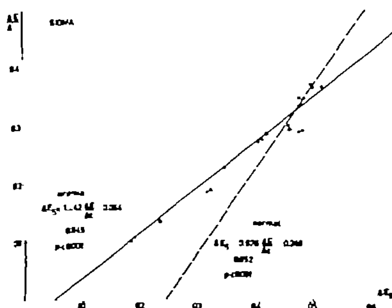


Figure 5

Linear correlation between $\frac{\Delta E}{\Delta t}$ and ΔE_s with the collagen preparation of Sigma

—— uremic patients

—— normal individuals

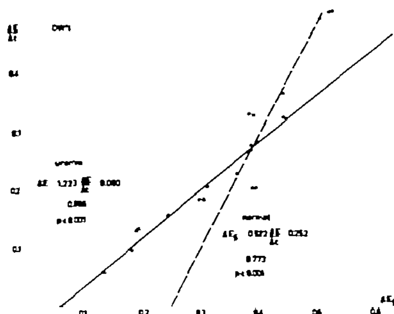


Figure 6

Linear correlation bet een $\frac{\Delta E}{\Delta t}$ and ΔE_s with our own collagen preparation

—— uremic patients

—— normal individuals

optical density obtained after adding Sigma collagen

Indeed no significant increase of initial optical density occurs after adding our own collagen preparation This can be explained by the smaller volume of collagen needed to obtain maximal platelet aggregation (30 µl own collagen preparation versus 0 1 ml collagen Sigma)

– A good linear correlation between the slope $\frac{\Delta E}{\Delta t}$ and ΔE_4 is found with both collagen preparations (Figures 5-6)

Interestingly a difference exists between the uremic patient group and the normal individuals. This may be explained by a somewhat different mechanism of platelet aggregation by collagen in the two groups. One can postulate that not only the rate of ADP release from platelets by collagen is faster in normal individuals than in uremic patients but that also other aggregating substances are released in a different way

Now the question to be answered is "which of the two collagen preparations used is the best one ? "

1 The difference between the means of the reaction times of uremic patients versus normal individuals is greater using the collagen preparation of Sigma Less overlapping between normal individuals and uremic patients is found with ΔE_4 as parameter when collagen of Sigma is used.

2. In four uremic patients the collagen aggregation with the Sigma preparation was grossly disturbed. With our own collagen preparation subnormal values were obtained. Three of these patients had an overt bleeding symptomatology So in these cases a better correlation between the results of platelet aggregation with the collagen preparation of Sigma and the clinical condition was obtained.

3 In some cases, not included in the groups presented here we have observed that the aggregation with collagen of Sigma was grossly disturbed, even in normal individuals, when the test was performed several hours after the PRP was prepared. With our own collagen preparation no changes were found. This probably reflects a greater sensitivity of the collagen preparation of Sigma to minor metabolic changes of the platelets.

In conclusion it appears that conflicting results obtained by different authors can easily be explained by the dissimilar aggregating capacity of the collagen preparations used.

By comparing the results of platelet aggregation in normal individuals and uremic patients it is possible to investigate the sensitivity of different collagen preparations

Acknowledgements

The authors wish to thank the Department of Nephrology and the Out-patient Unit of Internal Medicine for help in providing the blood samples.

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DISCUSSION

J HUGUES I completely agree with Dr Legrand and I don t have very much to add.

In our laboratory we use purified collagen soluble in a neutral medium. The collagen is prepared by Ch. M Lapière from rat and guinea pig skin according to the technique described by Van Caneghem and Lapière (1) It contains between 8.5 and 8.8 per cent hydroxyproline residues. The ratio between hydroxyproline and proline is constant 1 2.

Our results with these collagens are quite reproducible when working with the same PRP on the same day With collagen extracted from guinea pigs, the mean optical density variation is 107 with a standard deviation of about 8. With rat skin collagen the mean optical density variation is 92.5 with a standard deviation of 4 7 The initial slope of the curve (77 7°) is very constant with a standard deviation of only 1 7° The duration of the latency period is more prolonged with rat collagen When using the same batch of collagen (stored at 4 C) on different PRP and over a long period of time (18 months) the standard deviation of course increases. The mean optical density variation with a mixture consisting of 1.2 ml PRP adjusted to 300,000 platelets/cmm + 1 2 ml buffer is 85 ± 13

We have also used a commercial collagen preparation extracted in an acid medium. Working repeatedly with PRP from the same control subject for 2 months we obtained very large variations in duration of lag period, the slope of the curve and the maximum optical density variation

J R. O BRIEN If it is felt that soluble collagen is a reagent giving more reproducible results I would like to know where I could buy some soluble collagen which will always give the same results.

J CAEN A soluble collagen is actually sold for the platelet aggregation test and it is controlled in our laboratory for Stago Paris.

H HOLMSEN We have used the same collagen preparation for three years now and it works very fine It consists of Sigma collagen which was solubilized in 0 1 % acetic acid we stored this at minus 60 C in small tubes and take only one tube at one time when we need it and it works very reproducibly (Biochim Biophys. Acta 186 254 1969)

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Question N 8

IS AGGREGATION BY COLLAGEN AND THROMBIN THE CONSEQUENCE OF ADP RELEASE ONLY ?

DISCUSSION

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G de GAETANO
J CAEN
H HOLMSEN
S CRONBERG
J HUGUES
F MICHAL

21 SOME EVIDENCE THAT ADP IS NOT THE ONLY AGENT RESPONSIBLE FOR COLLAGEN INDUCED AGGREGATION

J Hugues

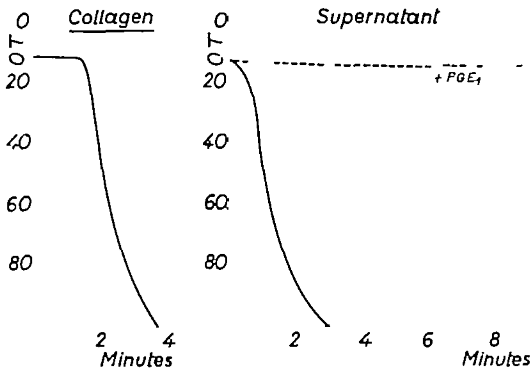
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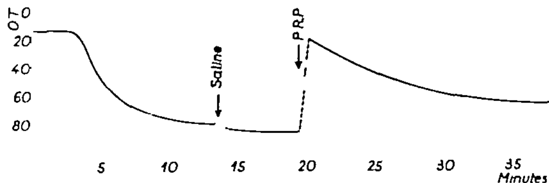
The role of ADP in collagen induced platelet aggregation has been suggested by Hovig (1) who demonstrated the release of platelet nucleotides in response to the action of collagen. Figure 1 illustrates a similar experiment. After exposure to collagen, the PRP supernatant is placed in contact with a new PRP and immediately clumps the new platelets. The fact that no new clumping takes place in the presence of PGE suggests that this action is actually due to the release of ADP (Figure 1). However the following data obtained during platelet aggregation by collagen do not support the concept that ADP is the only factor in collagen-induced clumping. Specific antagonists of ADP such as adenosine dichloroadenosine and chiefly PGE are only very slight inhibitors of the action of collagen (Table I). Likewise EDTA and promethazine disperse the clumps formed by ADP more rapidly and more efficiently than those induced by collagen Collagen induced clumps rarely disperse and when they do dispersion is much slower than in aggregates induced by ADP When an additional amount of PRP is added to the tube after the reaction between platelets and collagen has reached completion aggregation of the newly added platelets is obtained. Precisely the same thing is observed if PGE_1 is added to the cuvette before the addition of the new PRP Thus the new aggregation curve cannot be attributed to ADP (Figure 2). We saw yesterday that with Glanzmann platelets there is some difference between the action of ADP and collagen We also saw that the early ultrastructural changes of platelets differ depending on whether they are exposed to ADP or collagen the main difference is the presence of glycogen granules in the microvesicles upon exposure to collagen but not to ADP We shall see later in connection with the mechanism of cyclic AMP that marked

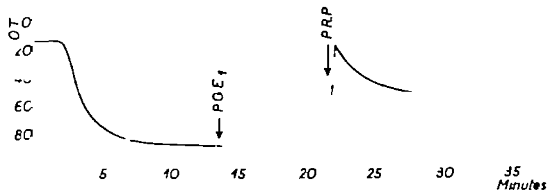
Table I

Effect of some inhibitors on platelet aggregation by collagen and ADP

Inhibitors	%ΔO D Collagen	ADP
Normal Platelets	100	100
Adenosine	95	76
Dichloroadenosine	98	22
PGE_1	94	10
Aspirin	87	74
Butazolidine	97	11
Persantin	109	110


Collagen
Supernatant
OT
0
20
40
60
80
+PGE1
2
4
6
8
Minutes


OT
0
20
40
60
80
Saline
PRP
5
10
15
20
25
30
35
Minutes


OT
0
80
PGE1
PRP
5
10
15
20
25
30
35
Minutes

differences also exist between collagen and ADP In conclusion it may be asserted that ADP is not the sole aggregating agent responsible for collagen-induced platelet clumping. Other factors may be involved such as the collagen itself or another as yet unknown substance released from the platlets or activated on their surface after contact with the protein.

REFERENCE

1 Hovig, T Release of platelet aggregating substance (adenosine diphosphate) from rabbit blood platelets induced by saline extract f tendons *Thrombos, Diathes. haemorrh* 2, 264 1963.

Figure 1

Left part PRP 1 2 ml + buffered saline (B S) 1 2 ml. At ero time addition f 0 8 mg sol ble collagen in 0 2 ml 0 4 M NaCL

Right part. PRP 1 2 ml + B.S. 1.2 ml At zero time addition f 0 2 ml supernatant of platelets previously aggregated by collagen.

—— normal diluted PRP

— diluted PRP incubated during 5 ith PGE_1 (0 5 mgr for 1.2 ml PRP)

Figure 2

Upper part

—— PRP 1 2 ml + B S 1 2 ml

At zero time addition of ADP (final concentration 10^{-6} M

— idem PRP previously incubated during 5' with PGE_1 (0.5 μg for 1.2 ml PRP).

Lower part

—— PRP 1 2 ml + B S 1.2 ml

At zero time addition of 5 μg adrenaline

— Idem. PRP previously incubated during 5' with PGE (0 5 μg per 1 ml PRP).

DISCUSSION

J.R. O BRIEN As far as thrombin is concerned, we are all agreed that thrombin can produce a double wave of aggregation If the second wave is associated with the release of ADP the first wave must be due to something else maybe an immediate effect of thrombin on the membrane I do not think the experiments with phospho-enol pyruvate and pyruvate kinase exclude this thus I think that the immediate action of thrombin is due to something other than release

I have studied aggregation induced by crude tendon extract, here called collagen and believe that the normal release reaction is auto-catalytic and that the reaction begins slowly after the delay and rapidly increases in speed up to a maximum, and that aggregation always proceeds to completion Recently I have found four other different kinds of response to collagen, and these kinds of response can be obtained from apparently normal people or from people on aspirin (Figure 1) A shows no response at all. B shows a decrease in amplitude indicating shape change but no aggregation. C shows partial aggregation and no shape change D shows shape change and aggregation but neither C or D ever showed complete aggregation Do any of these tracings indicate partial release if such a phenomenon is possible

G de GAETANO Aggregation by both collagen and adrenaline *can* be *partially* inhibited by anti-inflammatory agents (aspirin indomethacin) added in vitro or in vivo In particular the second wave induced by adrenaline can be partially inhibited. This suggests that the release reaction is not an "all or nothing" phenomenon.

H. HOLMSEN The experiments published by Haslam (2) in which washed platelets were incubated with thrombin and the aggregation was inhibited by the PK/PEP system, were discussed elsewhere and it is quite clear now that a small degree of aggregation does take place Thomas and Niewiarowski used a simpler technique employing a magnifying glass, and, by looking at what happens in the aggregometer they observed that there is a considerable aggregation by thrombin, even in the presence of an ADP removing system. The same is true with collagen moreover adrenaline aggregates platelets without release and ADP

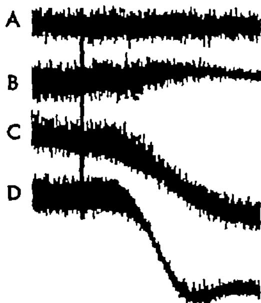


Figure 1

Four different kinds of response of PRP to collagen. For the explanation, see text.

also (first wave). It appears that all compounds which aggregate platelets have a specific aggre gating effect, which might be small but is completely independent on ADP

J CAEN There is agreement between dif ferent speakers that collagen acts through ADP release but also by other means. Has anybody an idea what these other means could be ?

G de GAETANO I would like to add in agreement with Dr Holmsen, that also purified bovine fibrinogen has a double effect on human platelets a first one nearly independent of ADP and a second one which seems to be related to ADP release I think that we should consider the possibility of some immunologic phenomena in the mechanism of action of bovine fibrinogen or of collagen preparations obtained from species different than man.

H HOLMSEN Solum has shown very clearly that bovine fibrinogen aggregates platelets in the presence of EDTA (3) He also observed that ADP is not released during the first wave of this aggregation. Since I am working with the release reaction it appears to me that all these agents which produce release in the end have to get the platelets to aggregate in order to induce release so perhaps the aggregated platelets are the real release stimulus (*Ser Haematol. 3* (IV) in press, 1971).

J.R. O'BRIEN May I repeat that I think propinquity of one platelet to another *however induced* (e g. centrifugation or aggregation) is the trigger mechanism inducing release

J CAEN Dr Holmsen how do you explain that thrombasthenic platelets which do not aggregate at all, do release perfectly well with collagen and bovine fibrinogen ?

H. HOLMSEN I agree that this is a tricky point to come around One could guess the following about thrombasthenic platelets they lack those substances in the membrane on which the charges are changed in order to get the cells aggregated. Otherwise they react quite normally It is shown that they have a normal content of thrombosthenin they have all these ingredients which probably are involved in both aggregation and release but not that part of the platelet surface which is necessary to get aggregation.

S CRONBERG When I first worked with thrombasthenic platelets and used a crude connective tissue suspension I saw nothing or little of this small aggregation but when working with the more pure and finely dispersed collagen of Dr Legrand we often saw a slight aggregation as demonstrated by Dr Hugues. As Dr Hardisty suggested I also think that this aggregation of thrombasthenic plate lets by collagen might be an adhesion to fine collagen fibres and not a real aggregation in the true sense This adhesion will induce release (1)

J R. O BRIEN Dr Hugues, can you explain figure 1 only when I added collagen submaximal aggregation occurred

J HUGUES Our technical approach is as follows. Prior to the addition of an aggregating agent, the PRP is always set at 10 per cent optical transmission. This means that the PPP does not always represent 100 per cent optical transmission It is therefore to be expected that the curve will not descend to the absolute bottom of the graph.

F MICHAL I agree with Dr Holmsen that platelets can aggregate without necessarily releasing adenosine diphosphate I have evidence that 5-hydroxytryptamine-induced aggregation does not necessarily go via the ADP pathway moreover 2 methylthioadenosine monophosphate which is a potent inhibitor of ADP induced aggregation does not antagonize aggregation induced by 5-hydroxytryptamine I would also agree with Dr O Brien that thrombin aggregates in at least two phases. With my instrument for measuring light scattering, I can differentiate 3 phases first there is a rapid reaction which we associate with platelet shape

change followed by a second step which is aggregation Often but not always this aggregation is followed by the third phase of irreversible aggregation With collagen, the rapid shape change (at least with the preparations I use) is also obvious

Dr Hugues's slide had adenosine and di-chloroadenosine listed as specific inhibitors of adenosine-diphosphate induced aggregation. Firstly the specificity of the inhibition of ADP-induced aggregation by adenosine and 2-chloroadenosine could be questioned because these substances inhibit aggregation caused by a variety of agents, unlike the nucelotide 2-methylthio-AMP which is a specific inhibitor of ADP Secondly di-chloroadenosine is not the correct name for the substance used by Dr Hugues it does not contain two chlorines but one chlorine substituted in the 2-position the correct name is really 2-chloroadenosine

J HUGUES The chemical structure is without big importance for me the importance is that this inhibitor acts on ADP but not on collagen-induced aggregation this is very important.

J CAEN With the first contrast microscope in our hands, at least 6-7 years ago we have found with S. Inceman that thrombasthenic platelets behave completely normally except the fact that there is no aggregation there is quite a normal so-called viscous metamorphosis of isolated platelets, in presence of thrombin and collagen.

J HUGUES For us it is quite easy to make the differentiation between adhesion to collagen fibers and aggregation in phase microscopy and electron microscopy we get aggregation even if very small, of Glanzmann platelets by collagen fibers in addition to adhesion.

J CAEN In the presence of collagen, have you done the experiment with calcium in order to precise the changes shown 12 years ago by Setna and Rosenthal and by Sharp also in thrombasthenic platelets ? I believe that aggregation is not necessary for a normal release

J HUGUES I have not done this experiment.

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Question N 9

DO PLATELET ANTIBODIES PROVOKE OR MODIFY THE RELEASE REACTION ?

DISCUSSION

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G V R. BORN
J R. O BRIEN
H. HOLMSEN
J HUGUES
G de GAETANO
J CAEN
R. GROSS
A SHARP

22. IMMUNE COMPLEXES AND PLATELET AGGREGATION

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The effect of antigen antibody complexes on platelets is known for considerable time already and so is the fact, that thereby it is unnecessary that the antigen involved cross-reacts with platelet constituents. Accordingly the same effect is observed with aggregated gammaglobulin (1,2) and with gammaglobulin adsorbed to certain particulate materials, such as latex particles (3) In contact with all these agents, platelets *show a release reaction* and provided calcium ions are present, progressive aggregation and contraction of the formed aggregates. For release alone Ca^{2+} ions are not required in fact it is observed even in the presence of EDTA.

Treatment of aggregated IgG at pH 4 destroys its platelet-aggregating properties, and aggregated IgA also proves inactive This points very strongly to a close relationship of the effect on platelets and complement (C) activation Human platelets, however other than for instance those of the rabbit, are still reactive in a fully synthetic suspension medium, i e in the absence of added C Mueller Eckhardt (2) has furthermore shown that the pretreatment of washed human platelets with chymotrypsin does not influence their reactivity towards immune complexes and, finally the fact that a release reaction takes place even in presence of EDTA, speaks very much against the involvement of the classical type of C-activation, which depends on Ca^{2+} and Mg^{2+} There can be no doubt that on the side of the gammaglobulin rather stringent structural requirements must be fulfilled for the acquirement of platelet-activating capacity This is illustrated best by a comparison of the effects of different aggregating agents on the activities of the resulting products (Pfueller unpublished data Table I). First it is evident that the fact that aggregation of γ-globulin occurs, does by no means imply the formation of an active product. Thus, aggregates obtained by trichloracetic acid are perfectly inactive those formed with dioxane show moderate activity as compared to the ones prepared by ethanol-precipitation. Secondly it is also evident that C-activating activity and effect on platelets as estimated by the release of ^{14}C-serotonin are generally in good agreement. This close relationship is further substantiated by experiments in which the free amino groups of the γ-globulin molecule were blocked by different agents. Again C-activation and the induction of release were affected simultaneously (Pfueller unpublished data) These last-mentioned results very strongly point to the importance of a certain specific charge distribution as a prerequisite for activity This lets immune complexes appear very much like collagen where it has been shown that the elimination of positively charged lysyl residues leads to the loss of aggregation-inducing capacity

Although closely related, th p

Table I

Effect of diff rent agents on human IgG
Given are gross effect of agent on gamma globulin, effect on complement (in percent) and on human platelets (in percent release of ^{14}C-labelled serotonin)

Solvent	Degree of γ-Globulin Aggregation	Compleme t Fixation at 38 μg/ml (%)	Serotoni Release t 200 μg/ml (%)
Acetone	+++	70	76
Ethanol	++	68	80
Dioxane	++++	15	10-26
Dimethylsulfoxide	++	35	67
Dimethylformamide	+++	51	74
Glycerol	0	5	2
Methylcellosolve	+	35	73
Methanol	+	40	60
Diethyleneglycol	±	0	2
Trichloroacetic acid(5%)	++++	0	1
NaOH (5N)	++	0	0

C activation and the effect of γ-globulin complexes on platelets seem not to be quite the same Thus, removal of carbohydrate destroys the platelet but not the C-activating activity of IgG-complexes (Pfueller unpublished data). It certainly will be of considerable interest to know more about the mode of action involved At present it seems most likely that a certain charge distribution on the surface of these complexes, which must be highly specific, is involved Since the same seems to be true for collagen it is perhaps justified, to put both agents into the same group of inducers of platelet aggregation Both of them have a direct effect on each individual platelet, which is independent of bivalent ions, and which manifests itself in a rapid release reaction

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23 EFFECT OF ANTIPLATELET ANTIBODIES ON THE 5-HYDROXYTRYPTAMINE UPTAKE BY BLOOD PLATELETS

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Treatment with antiplatelet antibodies (APA) reduces the in vitro uptake of serotonin (5-Hydroxytryptamine or 5-HT) by blood platelets (1 7) This phenomenon was used to evaluate the antiplatelet antibodies of a series of antilymphocyte sera (ALS) (4)

Methods

Blood platelets were isolated and washed by the method described by Weissbach et al (8) The technique of Lowry et al (5) was used for platelet protein determinations. Radioactivity measurements were performed in a liquid scintillation counter The APA were studied in 17 ALS of various origin and various activity (Table 1) Complement fixation and platelet agglutination were determined by the methods of Colombani et al (?) and of Dausset and Malinvaud (3)

The solution containing the platelet suspension (approximately 2×10^5 platelets/mm) deprived of leukocytes and erythrocytes, is isotonic and buffered at pH 7 35 The buffer solution contains, per litre NaHPO 16 24 mM NaH PO 3 76 mM KCl 4 00 mM ethylenediaminetetraacetic acid-Na_2 ..69 mM glucose 5.55 mM $NaCH_3COO$ 11 03 mM and NaCl, 104.54 mM Of this preparation 0.5 ml is mixed with 0 ? ml of ALS (diluted if necessary in isotonic saline) and incubated for 90 min at 37°C with mechanical agitation Two-tenths of a milliliter of a solution [illegible] C 5-HT in isotonic saline is then added The incubation is prolonged for 90 min more The preparation is then centrifuged The platelet sediment is washed twice with cold isotonic saline and dissolved with 1 ml of 0 0 N HCl for determination of protein content and radioactivity The inhibition of 5-HT uptake is expressed as a percentage of the uptake observed in control series where ALS is replaced by normal horse serum

Results

Definition of an APA Unit

We suggest that a unit of antiplatelet antibody (APA) be defined as the amount of APA which inhibits 50% of the 5-HT uptake by 4.8×10^8 platelets (corresponding to 1 mg of platelet protein)

For example one of the ALS studied in our series (and used by us as reference sample No 90606) inhibits 50% of the 5-HT uptake by 7.49×10^8 platelets (1 56 mg of platelet protein) Since the 0.2 ml of ALS used in the test is 4 times diluted the titre of this ALS may be expressed as 31 APA units/ml (Figures 1 and)

Table I

A study of anti platelet antibodies in 17 antihuman horse ALS.

Sample No	Horse No	ALS	Antigen used for ALS preparation	Complement fixation (reciprocal titre)	Platelet agglutination (reciprocal titre)	Inhibition of 5-HT uptake by platelet (units/ml)	In vivo thrombopenic effect (if tested)
1	341	LH0 1	Blood lymphocytes	256	64	169	
	379	LH018	Blood lymphocytes	128	32	65	
3	298	90583	Blood lymphocytes	128	64	61	+
4	298	90623[a]	Blood lymphocytes	128	128	39	
5	343-344	LH019	Blood lymphocytes	128	32	39	
6	298	90622	Blood lymphocytes	128	128	35	
7	298	90606	Blood lymphocytes	128	64	31	+
8	329	LH001	Blood lymphocytes	128	3	26	
9	329	90537A	Blood lymphocytes	128	32	[illegible]2	+
10	335-338	LH023	Spleen and blood lymphos	16	8	16	
11	335	90549	Spleen	2	2	15	0
12	338	90553	Spleen	Negative	2	11	0
13	337	90552	Spleen	4	16	10	0
14	336	90551	Spleen	Negative	1	8	0
15	340	LH020	Leukemia blood lymphocytes	Negative	Negative	7	
16	339	90544	Thymus	Negative	Negative	4	0
17	339	LH022	Thymus	4		4	0

[a] Antilymphocyte IgG

Table II

Inhibition of 5-HT uptake (μ moles/g protein) by blood platelets at various concentrations of extracellular 5-HT (μ moles/l)

APA units	5-HT uptake A	5-HT uptake B	Percentage of inhibition A	Percentage of inhibition B
0	9 09	5 19	0	0
0.39	6 66	3 0	7	29
0 78	5 93	96	35	43
1.55	5 1	41	43	54
3 10	4 45	53	51	51

Table III

Inhibition of 5-HT uptake (μ moles/g protein) by blood platelets at various concentrations of APA

A Extracellular ^{14}C 5-HT 44 4 x 10^{-4} M

B Extracellular ^{14}C 5-HT 22 2 x 10^{-4} M

Extracellular ^{14}C 5-HT	5-HT uptake Without APA	5-HT uptake With APA	Percentage of Inhibition
38	3 12	1 45	55
15	3 75	1 76	53
228	4.28	2.17	49
304	4 69	2.27	5

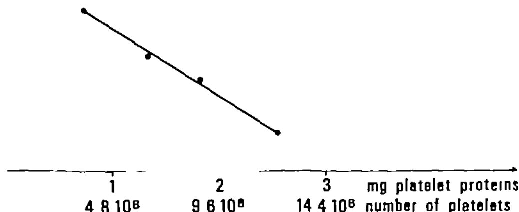


Figure 1

Measurement of antiplatelet ant bodies in ALS sample 90606 Th 5-HT uptak inhibition induced by 0 2 ml of the ALS, is studied on various concentrations f platelet The amount of 2 C 5-HT in the inc bation medium is constant (12 14 10^{-4} M)

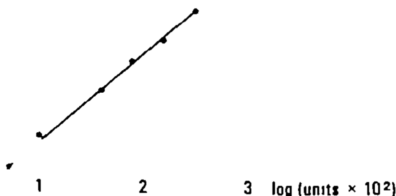


Figure 2

Standard slope btained with ALS sample 90606 The percentage f 5-HT uptake inhibition (bscissa) plotted versus the logarithm of APA unit X 10^2 (ordinat) Number of platelet 0 85 x 10 L tracefloLr 2 C 5-HT concentration 15 17 10^{-4} M.

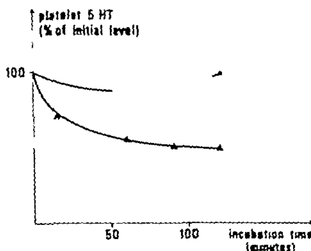


Figure 3

The 5-HT outflow f m platelet t the ext acellular medium. Following 120-min incubation w th 54 6 10 M F 2 14C 5-HT 1 82 10 platelet (or 0 36 mg of platelet pr tein) ar incubated without 5-HT Result are expressed as perce tage of the 5-HT intracellula concent ation which had been measured t the beginning f the incubation without 5-HT Circles represent an experiment without APA Triangles represent an experiment with 1 72 units of APA/ml

Effect of Extra-cellular 5-HT Concentration

The percentage of inhibition is independent of the 5-HT concentration in the incubation solution (Table II) This was found with what ever the amount of APA (Table III) in other words, the degree of inhibition is independent of extracellular 5-HT concentration

Since the 5 HT passive diffusion into blood platelets is normally proportional to 5-HT extracellular concentration it may be con luded that APA inhib t this passive diffusion

Relation between the Amount of APA and the Degree f Inhibition

The in vitro inh b tion of 5 HT uptake by blood platelets has been studied with various dilutions of ALS ompared w th the reference sample of ALS (No 90606) Within the limits of 10-80% inhibition is proportional to the logarithm of the amount of APA int odu ed into the incubation solution (Figure)

Inhibition of 5-HT Storage

It may be seen (Figure 3) that APA also inhibits the storage of 5 HT in platelets. This storage inhibition is probably explained by alterations of the platelet energetic metabolism, as described by Marcacci et al (6)

The above results suggest that the effect of APA is on one hand a modification of the active 5 HT uptake (responsible for the storage) and on the other hand, an alteration of the passive diffusion of 5-HT into blood platelets.

Acknowledgement

W wish t thank the Ed tor of *Transplantation* for giving u the permission t reproduc figures N 1 2 d 3

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24 A SPECIFIC HUMAN IMMUNOGLOBULIN G PREPARATION CAUSING PLATELET CLUMPING THROUGH A RELEASE REACTION*

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The formation of platelet clumps by isologous platelet antibodies has been observed repeatedly the antibody being most frequently found in the serum of polytransfused patients (3 8). It is generally accepted that these antibodies act as agglutinins. We have described a human immunoglobulin preparation which produces platelet clumping by a different mechanism (4).

This platelet clumping activity was found in plasma and serum from a patient with severe chronic idiopathic thrombocytopenic purpura, who had been transfused several years previously with platelet rich plasma because of post-partum haemorrhage In addition, she had again received large amounts of platelet-rich plasma during and after surgical intervention for a subdural haematoma with no "in vivo" recovery One week later the first test for platelet clumping activity was performed

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On leave of absence from the Laboratory of Blood Coagulation (Prof Dr. B. Bizzi), Department of Internal Medicine (Prof. Dr R. Bruda) Catholic University Roma, Italy Fellowship of the Ministero della Pubblica Istruzione of Italy 1970 and 1971

Figure 1 shows the effect of the addition of different dilutions of serum of the patient to a normal platelet-rich plasma in Born s platelet aggregometer (1) After a latent period sudden and very marked platelet clumping occurs. The duration of the latent period is inversely related to the amount of platelet-poor plasma or serum of the patient added The tangent to the steepest slope in the light transmittance records, expressing the rate of platelet clumping (6) is directly related to the amount of platelet-poor plasma or serum of the patient added.

Despite splenectomy the platelet count of the patient remained unchanged. Subsequent treatment, first with azathioprine (to 00 mg/day) later with the association azathioprine-prednisone failed to produce an increase in platelet number In contrast to this, the platelet clumping activity disappeared from the patient s serum, being no longer observed a few months after splenectomy

Platelet rich plasma from about 80 % of the volunteers of blood group O tested, underwent similar changes after addition of platelet-poor plasma or serum of the patient and will hence be termed "responsive" In plasma from 70 % of otherwise normal subjects no platelet clum-

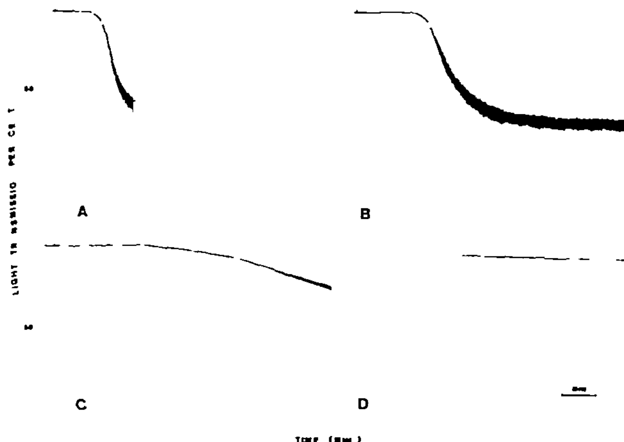


Figure 1

Addition of 50 microliter of isotonic saline dilutions of the serum of the patient to 0.95 ml normal citrated platelet-rich plasma. A dilution 1 B dilution 2x. C dilution 4x. D dilution 8x

ping occurred (unresponsive" subjects). None of these persons had taken drugs during the previous weeks.

The properties of this clumping activity are summarized in Table I

Evidence that clumping activity in the patient s serum is of an immunological nature is indicated in Table II

Evidence that clumping by immunoglobulin G is mediated by the release of endogenous platelet ADP was provided by the experiments summarized in Table III

It was thought that the immunoglobulin may produce lysis of a few platelets and that liberation of platelet constituents may lead to aggregation of the remaining platelets. Therefore we studied the effect of a normal platelet lysate (produced by repeated freezing and thawing of a suspension of washed platelets) on platelet aggregation. Whereas this lysate did produce immediate clumping in platelet-rich plasma, it had, contrary to the immunoglobulin preparation no effect on a suspension of washed platelets. Therefore the effect of the patient s immunoglobulin preparation cannot simply be ascribed to the lysis of substrate platelets. It was also observed that the patient s plasma or serum were able to make available platelet factor 3 (PF 3) as measured by the Stypven time PF 3 was found to be made available independently of platelet clumping (see paper n. 16 of this conference). Figure 4

Table I

Properties of the clumping activity in the patient plasma

1. Present in plasma and serum
2. Not dialyzable
3. Resistant to heating at 56 C for 30 minutes
4. Confined to the IgG protein fraction
5. Capable of clumping extensively washed human platelets
6. Produces an increase of platelet factor 3 activity which precedes the onset of platelet clumping
7. Rate of clumping is temperature-dependent.

Table II

Evidence that clumping activity in the patient' serum is of an immunologic nature

1. Observed after repeated transfusions of isologous platelets
2. The existence of "responders" and "non-responders" in normal population
3. Exhaustion of clumping activity by previous exposure to "responsive" platelet
4. Its presence in the IgG fraction only

Table III

Evidence that clumping by the patient immunoglobulin is mediated through release of platelet ADP

1. Increase of plasma ADP sufficient to provoke aggregation
2. Inhibitors of ADP-induced aggregation inhibit clumping by immunoglobulin
   - adenosine
   - Na_2 EDTA
   - TAM
   - PK-PEP system
   - 2-deoxy-D-glucose and antimycin A
3. Inhibitors of ADP-release inhibit clumping by immunoglobulin
   - acetylsalicylic acid
   - indomethacin
   - heparin (?)

shows the results of PF 3 determinations on platelet rich plasma from one "responsive" and one "unresponsive" subject, performed with different samples of the patient s serum. It can be seen that a very pronounced increase of PF 3 availability was observed during the first minutes after addition of the patient s serum. This activity was present in the patient s serum, obtained before and just after splenectomy and disappeared four months later platelets from the "unresponsive" subjects liberated PF 3 only in presence of the patient s serum obtained before splenectomy but to a minor degree as compared with "responsive" subjects.

Inhibition of clot retraction (9) by the patient s plasma or serum was only observed in "responsive" platelet-rich plasma. The degree of inhibition was variable for the different subjects studied and did not run in parallel neither with clumping activity nor with PF 3 availability. This inhibitory activity was generally much stronger in samples collected before or few days after splenectomy and was never observed in those obtained 4 months after operation.

The complement fixation test (2) was also found to be positive in the presence of "responsive" platelets and patient s serum the behaviour of this test was nearly identical to the results of inhibition of clot retraction. In some subjects, however complement fixation was observed also with patient s samples collected more than four months after splenec tomy when all other tests were negative.

These results suggest that complement is involved in the interaction of platelets with the patient s immunoglobulin however the observation that the purified immunoglobulin can clump extensively washed platelets is in contradiction with plasma complement playing an essential part in this reaction this conclusion is in agreement with the observations reported by Mueller Eckhardt and Lüscher (5) and by Pariyananda and Mowbray (7).

In addition, we found that sera from other patients with chronic idiopathic thrombocytopenic purpura, which were capable of inhibiting clot retraction or fixing complement in the presence of normal platelets, could not

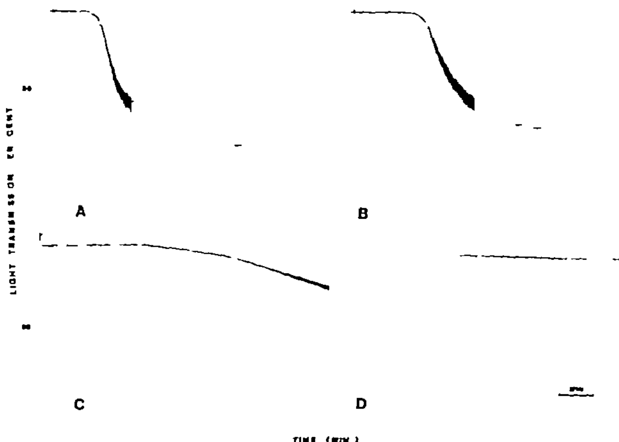


Figure 1

Addition of 50 microliter of isotonic saline dilutions f the serum of the patient to 0 95 ml normal citrated platelet-rich plasma A dilution 1x. B dilution 2x. C dilution 4x D dilution 8x.

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DISCUSSION

R.M. HARDISTY: I want to ask Dr Luscher when he talks about "release reaction" in this context does he mean the same as the release induced by collagen or thrombin in other words, do the nucleotides that are released come from the storage pool or is this a more non-specific damage such as might be expected if there was actual damage to the platelet membrane perhaps by complement for example?

E.F. LUSCHER: I think one has to be very careful about that with a platelet-specific antibody and complement present in the plasma general damage to the cell membrane is to be expected. What I have been referring to here is the effect of unrelated immune complexes and aggregated gamma globulins. These undoubtedly induce release from the storage pool they compare with collagen and have nothing in common with regular complement damage to a cell membrane.

G.V.R. BORN: Dr Luscher are you releasing only non-metabolic nucleotides

E.F. LUSCHER: That is right

J.R. O BRIEN: Dr Luscher to what extent are these antibody complexes a special surface? Do they differ from precipitated albumin for exemple

E.F. LUSCHER: There is quite a striking difference aggregated albumin does do nothing.

H. HOLMSEN: What is the size of these aggregates, and does this particle size correlate to the optimal size of latex particles?

E.F. LUSCHER: There is no doubt that these particles may be much smaller in fact soluble complexes, as long as they have antibody in excess, show the same effect as insoluble complexes. It is rather interesting that soluble complexes in antigen excess do not work. The essential structure is provided by the gamma globulin molecule and most likely as one knows from complement activation by two gamma globulin molecules in close vicinity

J. HUGUES: May I mention a very recent paper by Salmon and Louis presented in Montreux which demonstrated that platelet factor 4 is released during intravascular clotting induced by intravenous injection of antigen-antibody complexes.

J.R. O BRIEN: I would like to ask Dr de Gaetano whether on adding the plasma or serum you mentioned this produced a shape change? Have you taken electron microscopic pictures of the aggregates induced by this material?

G. de GAETANO: We have not yet performed any study with the electron microscope but we have planned to do it in collaboration with Professor Hugues and Dr David in Liège so I do not know now if there is a shape change. I can say however that the latent period was followed immediately by aggregation without the transient decrease of light transmission which is sometimes observed with colla[illegible] Furthermore the immediate deve

platelet factor 3 activity following addition of the immunoglobulin suggests a very rapid modification of the platelet membrane

J.R. O BRIEN Do you think that this phenomenon which you have called "clumping" is a different process from ADP-induced aggregation or collagen-induced aggregation ?

G de GAETANO The International Committee on Haemostasis and Thrombosis has recommended that the term platelet "agglutination" be reserved for the clumping of platelets by anti-platelet antibodies. Strictly speaking therefore the phenomenon we have described could be considered as a form of agglutination. However as ADP is clearly involved in this phenomenon, the term "aggregation" could be used. We think that if we add some human immunoglobulin preparations to the list of the aggregating agents we could refer to the described phenomenon as "aggregation" In any case "clumping" is maybe the term to be used.

J CAEN Are calcium or magnesium necessary for aggregation in your system ?

G de GAETANO No they seem not to be necessary washed platelets being suspended only in isotonic saline However release of calcium ions from the platelets may occur

R GROSS I would agree with Dr de Gaetano *in using the term clumping because in our* experience there are rather different reactions some years ago we compared these reactions platelets from thrombasthenic patients as well as platelets from normal persons treated with mono-iodo-acetate did not aggregate by addition of thrombin and other substances (at this time we had no experience with ADP) immune sera after polytransfusion agglutinated or clumped these thrombasthenic or mono-iodo-acetate treated platelets.

G de GAETANO May I repeat that clumping by this immunoglobulin was inhibited by combined 2-deoxy-D-glucose and antimycin A ?

E.F LUSCHER I have shown before that, with immune complexes, the release reaction occurs in the absence of calcium and therefore is independent of the ADP pathway of aggregation With large quantities of inducing agents, a mixed agglutination reaction, based on mutual adherence of platelets to immune complexes may predominate with smaller amounts, aggregation most likely follows the release reaction and therefore the classical pathway of ADP-aggregation Depending on how much material is available both reactions with either one or the other type prevalent are possible The second point refers to the effect of heparin, which on Dr de Gaetano s slide carried a question mark. All the experiments to which I have been referring were done in the presence of hirudin and therefore it seems unlikely that coagulation plays any role since we have no evidence for a direct participation of complement, it seems unlikely again that heparin acts as an anticomplementary agent. Have you any idea as to a possible point of attack of heparin in this reaction ?

G de GAETANO It is possible that heparin could act as an inhibitor of the antigen-antibody reaction (1) we must consider however that Dr O Brien has shown that heparin is an inhibitor of collagen-induced aggregation (3) So we could consider heparin to have an action similar to aspirin or some other inhibitor of the "release reaction"

A.A SHARP I would like to ask Dr de Gaetano if his antibody works in the presence of EDTA I ask this question because last year there was a published report of a patient with a violent clumping phenomenon in EDTA blood it was thought from these studies that this was due to a powerful antiplatelet-antibody I too have seen the same phenomenon but the patient died before the phenomenon was properly investigated. It was, however very striking and I wonder if your material worked also on EDTA-platelet-rich plasma

G de GAETANO No our material does not

work on EDTA platelets and I think it is another argument in favour of release reaction and of ADP involvement in this clumping.

H HOLMSEN Hovig (2) showed that heparin in high quantities inhibits the aggregation by tendon extracts.

I have a comment with regard to Lüscher who said that the release reaction was independent of metal ions. We should remember that during the release reaction quite a lot of calcium is set free from the platelets. So if one is not working with EDTA plasma as dr Lüscher commented, platelet aggregation may just as well go through the classical ADP way

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Question N 10

HOW DOES ADENOSINE INHIBIT PLATELET AGGREGATION ?

25 H. HOLMSEN A "Transport Theory" for Inhibition of Platelet Aggregation by Adenosine
26. G V.R. BORN Role of the Competition in the Inhibition of Platelet Aggregation by Adenosine

DISCUSSION

H. HOLMSEN
S BYGDEMAN

G V.R. BORN
J W ten CATE

25 A "TRANSPORT THEORY" FOR INHIBITION OF PLATELET AGGREGATION BY ADENOSINE

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Figure 1 summarizes 3 hypotheses on the inhibition by adenosine of ADP-induced platelet aggregation which were current in 1968. The first one was proposed by Born (1) and states that adenosine and ADP can bind to one and the same receptor site whereby adenosine inhibits by competitive inhibition. The second was inferred by Salzman et al. (10) and based on their *ecto*-ATPase hypothesis for platelet aggregation: platelets have an *ecto*-ATPase which is *constantly consuming ATP from the* cell, and the energy released is linked to the process of keeping the platelet in an *unsticky* state. Then, if ADP is added it interferes with the ATPase by product inhibition and the ATPase is no longer capable to keep the platelet unsticky: it becomes sticky and aggregation occurs. When adenosine was taken up by the platelet, much ATP was produced from this adenosine near the membrane thus making more substrate for the *ecto*-ATPase. Then addition of ADP would not give the same effect as with platelets without elevation of the ATP level. Rozenberg and myself (8) studied the kinetics of adenosine metabolism in human platelet-rich plasma (PRP). There appeared to be a close connection between the uptake of adenosine and inhibition of aggregation and some hypotheses for the mode of action of adenosine were proposed: when adenosine is taken up, ATP is consumed in the adenosine kinase reaction (4) (in disagreement with Salzman et al.'s statement that it *produces* ATP) and this ATP-consumption competes with that pool of ATP which is utilized in platelet aggregation. The adenosine which has been taken up and converted to AMP could also

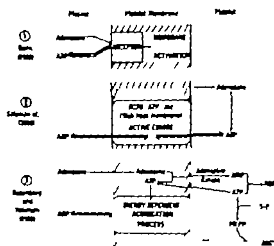


Figure 1

Hypotheses of action of ADP and adenosine on platelets. The figure was kindly provided by dr. R.J. Haslam.

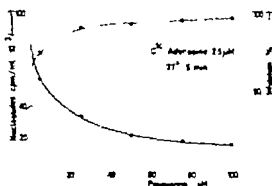


Figure 2

Effect of papaverine on formation of platelet nucleotides from C^{14}-adenosine (adenosine uptak) and on inhibition f aggregation Nucleotides (ATP + ADP + AMP) were extracted with 43,2% ethanol-5 mM EDTA, and their radioactivity determined as described elsewhere (4) Inhibition of platelet ggregation was measured as described previously (8) using 10^{-4}M ADP

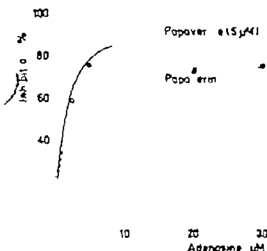


Figure 3

Effect of papaverine on adenosine-induced inhibition of platelet aggregation The aggregation wa induced by 10^{-4}M ADP and its inhibition with or without papaverine (5 min pre-incubation) was determined as described elsewhere (8)

interfere stereochemically with myokinase which in some way could inhibit ADP induced aggregation

Both Markwardt et al (5) as well as Born and Mills ( ) have shown that papaverine inhibits the uptake of adenosine w th a concomitant *increase* in the inhibition of platelet aggregation. This fact seemed incompatible with our hypothesis that inhibition was connected with the uptake of the riboside Never theless the following experiments with papaverine do not obviate the idea that transport of adenosine across the platelet membrane might indeed interfer with the aggregability of the cells. Figure 2 demonstrates the effect of increasing concentration of papaverine on adenosine uptake and inhibition of aggregation. The degree of inhibition is enhanced, whereas uptake of adenosine is decreased by increase of papaverine seemingly destroying any connec tion between uptake and inhibition Variation of the concentration of adenosine in the presence and absence of papaverine shows that the inhibition of aggregation reaches a saturating level at a certain adenosine concentration (Figure 3) Presence or absence of papaverine does not alter this saturating concentration It is only the *degree* of inhibition which is affected. As has been demonstrated earlier (8), the uptake of adenosine is also saturated at the same concentration of adenosine which produces maximal inhibition Similar to inhibition of platelet aggregation papaverine does not alter the saturation kinetics of adenosine uptake only the uptake rate.

Figure 4 shows the metabolism of adenosine in PRP with simultaneous measurement of inhibition of aggregation in the absence or presence of papaverine In the absence of *papaverine* adenosine reaches a low level after 30 minutes and the inhibition is abolished. If papaverine is present we create a situation with very little adenosine in the system, but con siderable inhibition of aggregation. Rozenberg and Ledwidge (9) have showed that addition of adenosine deaminase to a papaverine plus adenosine treated sample results in an inhibition of aggregation which lasted for 30 minutes at 37°C *with no adenosine in the system.*

Uptake and inhibition have the same saturation kinetics and papaverine or persantin only heighten the level of inhibition without changing the saturation kinetics. The experiments with papaverine and adenosine deaminase indi-

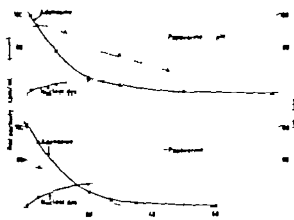


Figure 4

Correlation between the level of C^{14} adenosine and C^{14}-nucleotides (ATP + ADP + AMP) with the degree of inhibition of platelet aggregation (induced by 10^{-4}M ADP) at different times after addition of C^{14}-adenosine to PRP (2,5 mM adenosine at zero-time) The methods are described elsewhere (4,8).

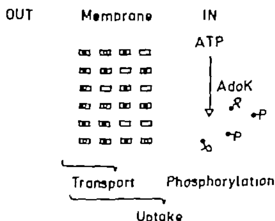


Figure 5

Transport of adenosine (0) across the platelet membrane facilitated by specific carrier substance. When released from the carrier complex at the inside of the membrane adenosine is phosphorylated by ATP catalyzed by adenosine kinase (AdoK)

cate that adenosine apparently binds to some thing in the membrane *before* it can exert its inhibitory action One hypothesis which could explain how adenosine can inhibit aggregation during its uptake is the "transport theory Figure 5 shows a unit membrane in which the quadrates indicate transport proteins for adenosine It has not been proved for platelets, but adenosine in many other cells (6,7 11) is transported with a transport protein or a carrier system across the membrane One can use this model to explain the inhibition of platelet aggregation by adenosine during its uptake Such a system works down a gradient (high concentration of adenosine on the outside of the membrane low concentration of adenosine on the inside of the membrane) and the function of the carrier is to facilitate this downhill movement of the hydrophilic adenosine The concentration at the inside of the membrane is kept low because adenosine kinase is present and phosphorylates adenosine immediately as it enters the cell Let us assume that the carrier adenosine complex in some way or other inhibits platelet aggregation, i.e the carrier complex is the inhibitory substance If adenosine deaminase is added to the outside it removes every adenosine molecule on the out side but high amounts of carrier-adenosine complexes remain in the membrane which explains why the inhibition lasts after a rapid removal of adenosine (8) Now we assume that substances like papaverine and persantin which slow down the uptake work by retarding the *movement* of the carrier complex across the membrane By removal of extracellular adenosine as Rozenberg and Ledwidge (9) did with adenosine deaminase a lot of these carrier adenosine molecules are left in the membrane but because they now move slowly *their inhibitory action lasts much longer* I do not know how to prove this theory but the amount of adenosine which is in the "transport state must be very small at least smaller than we have ever been able to detect with adenosine of the highest specific radioactivity available

The "transport theory" has been discussed in more detail elsewhere (3)

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26 ROLE OF THE COMPETITION IN INHIBITION OF PLATELET AGGREGATION BY ADENOSINE

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We have done experiments similar to those reported by Holmsen (2) with both papaverine and persantin (1) Because these drugs inhibit the uptake of adenosine their use should show whether the Holmsen's hypothesis (2) is in accordance with facts. Adenosine uptake could be completely inhibited without decrease in the inhibitory effect of adenosine on aggregation on the contrary it was potentiated. The potentiation has also been seen by Holmsen and needs explanation My view which is similar to Holmsen's and also expressed before is that the effect of adenosine must be in the membrane that seems to be agreed and is not so surprising because adenosine kinase is probably in the platelet membrane as in the membrane of other cells.

Just because an agonist molecule a molecule that does something, is charged and its antagonist molecule is not charged this does not necessarily mean that the two do not compete for the same receptor Thus, acetylcholine is a highly charged quaternary ammonium molecule and atropine not so charged acts competitively at the acetylcholine receptor

Nevertheless I agree with Holmsen that the best evidence is that adenosine does not act competitively at exactly the same site where ADP does its work Incidentally it is interesting that ATP and ? methylthio-AMP are inhibitory also with apparently competitive kinetics both are phosphorylated compounds. One could suggest therefore that ATP and -methylthio-AMP are direct competitive inhibitors.

Even in the initial shape change the adenosine inhibition is apparently competitive What one has to conclude is that, even if the system is not a simple sort of bi-molecule competition at a particular binding site there is a competitive element in the situation

I have always thought that there are two possibilities of developing this idea. One is competition for a third component. The other is that even if there is not a binding site for adenosine in the sense that there is a binding site for ADP there must still be a binding site because presumably adenosine kinase and adenosine deaminase have specific binding sites for their substrate In fact, it is a basic assumption in enzyme kinetics that they have such sites. Holmsen's idea that the effect of adenosine is caused by being bound to its kinase or to its transport system is one of several possibilities.

I think it will ultimately turn out that adenosine causes some allosteric change in a set of linked proteins by which their reactivity to ADP is diminished. Such an effect has still to be demonstrated.

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DISCUSSION

H HOLMSEN Dr Born I have a comment in support for our common theory The Bela march group (Boston University) has shown that the aggregation of thrombocytes from animals which does not occur with ADP but *only with thrombin is strongly inhibited by* adenosine

S BYGDEMAN In some recent experiments we have observed that addition of KCN or NaCN to platelet-rich plasma in a 0 01 M concentration can decrease the inhibitory effect of adenosine and AMP on ADP-induced platelet adhesiveness. The results are shown in Figure 1

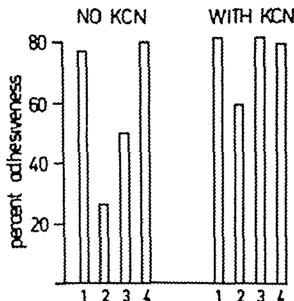


Figure 1

Effect of KCN on the inhibitory effect of adenosine and AMP on ADP-induced platelet adhesiveness. For details, see the text

Column 1 and 4 are control values Column 2 indicates platelet adhesiveness after addition of 1 μg/ml of adenosine and column 3 after addition of 1 μg/ml of AMP Preincubation with KCN markedly decreased the inhibitory *effect of AMP and adenosine Final concen*tration of ADP 0.3 μg/ml.

G V R BORN Could I ask how you measure stickiness ?

S BYGDEMAN With the Hellem method.

G V R BORN Is the AMP free of adenosine ?

S BYGDEMAN It was commercially available AMP manufactured by Sigma Similar results were obtained when ADP-induced platelet aggregation was studied. KCN and NaCN in a final concentration of 0 1 M reduced the inhibitory effect of adenosine and AMP on ADP-induced platelet aggregation At the same time however platelet uptake of adenosine was not diminished

H. HOLMSEN I do quite agree with Bygdeman when he says that KCN is not affecting platelet aggregation we saw on his last slide that there was a small increase with KCN of platelet aggregation induced by ADP and the change which was produced on the sample which was incubated with adenosine and KCN was very small I have therefore no comments on the data he is presenting here In addition the adhesion if I am not wrong, was 80 per cent and that is approximately the highest you can get So any effect of KCN as stimulator would not be revealed if you have that high

27 METHYL XANTINES IN THE INHIBITION OF PLATELET AGGREGATION

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Sutherland and coworkers (4) have in a series of reports demonstrated that an important regulating factor in the control of many cellular processes appears to be the intracellular level of adenosine 3' 5'-monophosphate cyclic AMP. The intracellular level of cyclic AMP depends on the activity of at least two enzymes, firstly adenyl cyclase which catalyzes the conversion of ATP to cyclic AMP and a phosphodiesterase which is responsible for the degradation of cyclic AMP to 5 AMP.

Several recent reports have demonstrated adenyl cyclase activity in platelets and it has been suggested that cyclic AMP may play a role in platelet adhesiveness and aggregation (3 5 6). It has thus been shown that prostaglandin E which stimulates platelet adenyl cyclase activity and the formation of cyclic AMP inhibits platelet aggregation while for instance noradrenaline which can induce platelet aggregation inhibits adenyl cyclase activity in the platelets (6). Ardlie et al. (1,2) have shown that methyl xanthines which inhibit phosphodiesterase activity and therefore can increase cyclic AMP level also counteracts ADP and catecholamine induced aggregation. In an attempt to

Table I

Effect of aminophylline and caffeine on adrenaline (A), noradrenaline (NA) and ADP induced aggregation. The figures indicate aggregation response in per cent of control (Mean values n = 5)

Methyl xantines		NA µg/ml			A µg/ml			ADP µg/ml		
		0.2	0.4	0.6	0.2	0.4	0.6	0.1	0.2	0.3
Aminophylline	10^{-3} M	21	35	33	87	94	80	69	74	81
concentration	5 10^{-3} M	0	0	0	6	9	9	16	36	37
	10^{-2} M	0	0	0	0	0	0	0	0	0
Caffeine	10^{-3} M	42	61	49	87	80	75	83	88	92
concentration	5 10^{-3} M	0	0	13	18	18	20	31	47	54
	10^{-2} M	0	0	0	0	0	0	0	0	0

evaluate the role of cyclic AMP in the regulation of different platelet functions we have in a pilot study tested the effect of methyl xantines on platelet aggregation and platelet uptake of ^{3}H-adenosine and ^{3}H-noradrenaline

The results obtained (Table I) show that methyl xantines inhibit ADP-and catecholamine-induced platelet aggregation and thus confirme the results reported by Ardlie et al. (1,2). More interesting however we observed that both caffeine and aminophylline in the same concentrations also markedly inhibited platelet uptake of ^{3}H-adenosine The degree of inhibition varied thus from 75 % at an aminophylline concentration of 10^{-2}M to 30 % when aminophylline was present in a concentration of 10^{-3}M In similar experiments an inhibition of the uptake of ^{3}H noradrenaline with 50 % and 18 % could also be observed after addition of aminophylline in a concentration of 10^{-2} and 10^{-3}M respectively If we assume that these effects of methyl xantines are secondary to an inhibition of phosphodiesterase activity this may indicate that cyclic AMP plays a regulatory role for many important platelet functions. However the high concentrations of methyl xantines necessary for an inhibition of phosphodiesterase activity always involves a risk for unspecific effects.

Acknowledgment

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DISCUSSION

H. JOHNSON Six different prostaglandins were investigated for inhibitory effect on ADP induced aggregation in platelet-rich plasma and were compared with the effect of adenosine. The prostaglandins were bio-synthesized by Upjohn Ltd. ADP was used in the final concentration of $1.56x10^{-6}$M. The same PRP was used in all tests. Aggregation was measured turbidometrically according to Born the inhibitory effect was measured as percent inhibition of control aggregation rate. PGE_1 had a 50 per cent inhibitory effect already at a concentration of 0.01 mcg per ml plasma, PGE_2 at 0.05 mcg per ml plasma therefore the inhibitory effect of PGE_2 was about only 20 per cent of that of PGE_1. PGE 217 had about the same effect as adenosine and shows an inhibitory effect at 0.5 mcg per ml plasma. The PGF 2 alpha and the PGF-1-beta first showed an inhibitory effect at 2 to 3 mcg per ml plasma whereas the PGF 1-alpha had no inhibitory effect at all. No stimulating effect on aggregation was found.

J. HUGUES There are three ways of modifying the activity of cyclic AMP inside a cell. The first is to add 3' 5' AMP or its dibutyryl derivative. The second is to inhibit or stimulate phosphodiesterase. The third is to inhibit adenyl cyclase.

Our experimental results are shown in Table I. The following conclusions may be drawn:

1. The results obtained with 3' 5' AMP, its dibutyryl derivative, methyl xanthines, papaverine and PGE in the presence of ADP and epinephrine are in good agreement with the cyclic AMP theory. Nevertheless it will be noted that the inhibitory concentrations used are quite high. Thus the possibility of an unspecific effect cannot be excluded.

2. If imidazol stimulates phosphodiesterase in platelets as it does in other cells, our findings are not consistent with the alleged role of cyclic AMP in platelet aggregation.

3. These inhibitors are often less potent in the presence of collagen than with ADP and epinephrine.

H. HOLMSEN Only a short comment on Dr Hugues statement that imidazol may not stimulate phosphodiesterase in platelets. The adenyl cyclase and phosphodiesterase systems are very different in different organs. Inhibitory in one organ, they might not necessarily be inhibitory in another. I think that Dr Mills has shown that imidazol is a potent inhibitor of phosphodiesterase.

S. BYGDEMAN I would like to ask if you have an idea about the effect of methyl xanthines. The concentrations necessary to inhibit phosphodiesterase activity are high and involve the risk of unspecific effects.

H. HOLMSEN I would suggest to Dr Bygdeman to contact Dr David Mills, because he studied the effect of methyl xanthines on the kinetics of inhibition of both adenyl cyclase and cyclic phosphodiesterase.

J. CAEN Do different concentrations of theophylline have the same action on the diesterase and on the various types of adenyl cyclases?

Table I

Inhibition of collagen- ADP and adrenaline-induced platelet aggregation by substances modifying cyclic AMP For every experiment, aggregation in presence of the inhibitor was calculated as percentage of control aggregation, taken as 100 per cent. The degree of inhibition was indicated as follows aggregation more than 95 per cent of control no inhibition ( ) between 91 and 95 % ± 76-90 % + 51 75 % ++ 26-50 % +++ 0-25 % ++++

Inhibitors	Final Concentration	Collagen	ADP	Adrenaline
3 5' AMP	10^{-3} M		+++	±
Dibutyryl 3 5 AMP	5×10^{-4} M	+	+++	++++
Caffeine	1 mM	–	–	
	10 mM	++++	++++	++++
Theophylline	1 mM		±	
	10 mM	++++	++++	++++
Papaverine	8×10^{-3} mM	+	+++	
Imidazol	12 mM	±	+	±
	120 mM	++++	++	++++
Gl cagon			–	
PGE_1	0.5 µg/ml	+	++++	++++

P.M MANNUCCI Has anybody any information on the action of nicotinic acid on phosphodiesterase or cyclic AMP as we have seen that nicotinic acid enhances the aggregation induced by ADP ?

H. HOLMSEN I can only refer to Dr Mills again he has worked with nicotinic acid Whether it inhibits or enhances the diesterase I do not remember but it has an effect on the enzyme activity

H HOLMSEN I have seen Dr Mills experiments on phosphodiesterase from ox heart cat brain and platelets the way these theophyllines affect the enzyme is entirely different among these preparations

G de GAETANO Has anybody any information whether cyclic AMP does act directly or as intracellular messenger which can regulate other reactions in the platelets ?

H HOLMSEN I think cyclic AMP in platelets acts as a regulator of platelet function According to dr Mills, it does not act as an intracellular inducer of aggregation because when ADP is added the decrease in the level of cyclic AMP is not of a magnitude that should be expected if it were the intracellular messenger

Question N 12

SIGNIFICANCE OF CONGENITAL OR ACQUIRED DEFECTIVE PLATELET AGGREGATION

DISCUSSION

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28 NEW PLATELET DISEASES

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Although instances of prolonged bleeding time unexplained by coagulopathy or thrombocytopenia have been known for some time the platelet dysfunction suspected in these cases has been difficult to investigate until recently However the clinical use of techniques testing accurately the platelet functions made it possible to screen some abnormalities. These techniques measure

- platelet "adhesiveness" obtained by letting blood flow through a glass bead column the index of "adhesiveness is in fact the result of several phenomena adhesion of platelets to glass beads, release and aggregation It takes into account not only the intrinsic properties of the platelets, but also the properties of the surrounding medium (3 10,18).
- variation in optical density of PRP during aggregation induced by several agents such as ADP collagen or adrenaline (2,15).
- availability of platelet factor 3 during aggregation (7,8 21)
- content of platelet components, mainly adenine nucleotides and 5-hydroxytryptamine (5HT) which are released during aggregation (14)

Since 1967 some investigators have described several patients suffering from mild bleeding disorders in whom various platelet dysfunctions were associated. Comparison between these diseases is sometimes difficult because of the lack of standardization of the techniques used.

Hardisty and Hutton (9) have described in some bleeders a fast disaggregation after addition of ADP and a diminished aggregation upon addition of thrombin noradrenaline and collagen. In these patients, platelet adhesiveness in vitro is reduced. Moreover availability of factor 3 induced by kaolin is weak although the total content of platelet factor 3 is normal.

A very particular thrombopathia was reported by Hirsch et al. (11) in a child suffering from spontaneous bruising and having a prolonged bleeding time Adhesion and aggregation to collagen were defective but release and factor 3 availability as well as ADP-aggregation appeared normal. It must be reminded that in thrombasthenia, adhesion to collagen is normal

In the thrombopathia observed by Weiss (22) in six women having various degrees of bleeding, aggregation by collagen and factor 3 activity were defective as well as platelet adhesiveness. The three abnormalities were corrected by addition of ADP An initial defective release of ADP was considered responsible for this state

O'Brien (16) has described ten patients with moderately prolonged bleeding time diminished adhesiveness and abnormal response to collagen and adrenaline

A familial platelet disease has been observed by Caen et al. (4) Three patients of the same family and a fourth patient showed a prolonged

bleeding time and a defective platelet adhesiveness. There were no response to collagen and a weak aggregation with adrenaline in the three former patients the lag-phase was prolonged and aggregation diminished upon addition of collagen in the fourth subject.

Sahud and Aggeler (19) have observed in a woman with prolonged bleeding time a mild bleeding symptomatology a normal factor 3 activity with kaolin in spite of a defective adhesion and aggregation upon addition of collagen Aggregation by adrenaline was also defective as well as aggregation by 5 HT Aggregation induced by ADP was normal, although disaggregation occurred very early at low concentrations. Moreover these workers described a normal aggregation by thrombin but a defective one by trypsin. Release of ADP was not measured

More recently studies on the content of platelet adenine nucleotides and 5 HT have brought some light on the nature of platelet diseases

An impaired release of ADP was observed by Weiss et al (25) in six members of a family whose bleeding time was slightly prolonged. In these patients, collagen induced the normal disk sphere transformation but, as a consequence of a defective release aggregation in presence of collagen and the second wave of epinephrine induced aggregation were absent Kaolin-induced platelet factor 3 activity was also impaired but corrected by ADP Primary aggregation by ADP was normal at room temperature but disaggregation was rapid at 37°C Adhesiveness was normal or diminished The decreased total content of ADP on platelets and their small size were suggestive of a population of prematurely senescent platelets It is of a great interest that the defect of these platelets and that induced by aspirin are additive wich suggests that the mechanism of these defects is different

In three members of a family affected by thrombopathia, Weiss and Holmsen (6) have shown a decrease in the storage pool of ADP whereas the metabolic pool appeared to be normal. During the release reaction induced by collagen, the degradation of some metabolic ATP to IMP and hypoxanthine was normal. It was therefore unlikely that the mechanism of the release was impaired.

In three cases of Wiskott Aldrich syndrome Gröttum et al (5) have observed a defective storage pool of adenine nucleotides. Platelet aggregation by ADP or collagen was low and adhesiveness was reduced Factor 3 activity with ADP or kaolin was diminished.

A defective storage of adenine nucleotides and a reduction of 5 HT uptake and content were also observed by Mills and Hardisty (13) in albinos with bleeding tendency Aggregation with collagen second wave of aggregation with other agents and factor 3 availability were strongly reduced.

Thus, some platelet diseases previously characterized by a diminished release seem more likely to be due to a defective uptake of adenine nucleotides and 5 HT In contrast with these diseases, abnormalities induced by aspirin are probably consecutive to a partial inhibition of the relase Aspirin inhibits the second phase of adrenaline-induced aggregation and decreases aggregation in presence of collagen never theless, platelet factor 3 activity is normal as well as platelet adhesiveness (17,23,24,27)

"New platelet diseases" seem to be different from other congenital disorders such as Glanzmann's thrombasthenia Von Willebrand's disease afibrinogenaemia or disease(s) with giant platelets (12). Glanzmann's thrombasthenia is at once identified by a deficient or absent clot retraction The inability of platelets to aggregate upon extrinsic ADP or upon ADP released by the platelets themselves, is a constant finding. However ADP can induce the normal shape changes from disk to spiny sphere Adhesion to collagen is nearly normal contrary to what is observed in the thrombopathia described by Hirsch et al. (11) The aggregation induced by collagen is reduced or nil The availability of platelet factor 3 appears to be diminished but not absent upon addition of ADP although there is no aggregation whatsoever Release was normal in two subjects

studied by us. Adhesiveness to glass beads is absent.

Von Willebrand's disease is characterized by a classic triad prolonged bleeding time low factor VIII and decreased platelet adhesiveness which appears only when the flow rate of whole blood through glass beads column is sufficiently high (3 18) This defective adhe siveness with native or slightly heparinized whole blood seems to be the only sign of platelet dysfunction. Aggregation, release and factor 3 availability are normal The clinical and biological defects of hemostasis are partially corrected by infusion of fresh plasma, hae mophilic plasma or cold precipitate The nature of the extra-platelet factor which promotes normal adhesiveness is presently uncertain. It may be identical to factor VIII or to its activator

In case of afibrinogenaemia the problem of the content of fibrinogen in platelets is still discussed and it is not sure that afibrinogenaemic platelets are completely devoid of fibrinogen. This point is of fundamental importance in the comprehension of the me chanism of aggregation.

In the "dystrophie thrombocytaire hémorragipare (1) or congenital thrombocytopenia with giant platelets (6) there is an important abnormality of platelet structure especially of platelet membrane which seems to correspond to the defect of factor 3 availability

Among the acquired conditions with bleeding tendency such as uraemia, fibrinolysis, leucosis, thrombocythaemia or dysglobulinaemia (12) *it is difficult to distinguish the* causes of platelet dysfunction because there are often several plasmatic abnormalities.

New platelet diseases are no doubt relatively frequent and seem often to correspond to an isolated bleeding state They provide models of great interest for the study of the mechanism of platelet functions and can help to better understand the complex aspects of hemostasis and thrombosis.

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29 RECENT DATA ON THROMBASTHENIA

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Thrombasthenia or Glanzmann disease is the most clearly defined disorder of haemostasis resulting from a congenital qualitative platelet abnormality and so it can serve as a useful model

But actually one does not know why the thrombasthenic platelets do not aggregate in the presence of ADP At phase microscopy as well as at electron microscopy In this disease at my knowledge the megakaryocytes behave normally

The platelet survival using either the patient s own platelets or compatible ones, is normal, excepted when one can detect an iso-antibody present in 3 out of our 22 patients.

When thrombasthenic platelets are separated by sucrose gradient by the method used by Booyse and Rafelson (2) one can find in this disease an abnormal pattern in the platelet profile with a decrease of the D population but a normal total platelet protein synthesis and a normal D-dependent and I-independent (of G-6-P) forms of glycogen synthetase activity So far it seems that in this disease we have an abnormal pattern of platelet populations. The question arises why ADP or any aggregating agent, excepted purified bovine fibrinogen, does not act on thrombasthenic platelets

1 As shown by Zucker (cited in 1) and also by ourselves (1) ADP brings a normal shape change of these platelets

2 Added to thrombasthenic platelet extracts before th ombin, ADP protects two lines, namely fibrinogen and platelet factor XIII against the thrombin action

3 When thrombasthenic platelet rich plasma is incubated with ADP prior to the addition of purified bovine fibrinogen ADP in contrast to epinephrine (this was done also recently in my laboratory by C onberg) is able to inhibit the "aggregating" or agglutinating effect of purified bovine fibrinogen on th ombasthenic plate lets

4 Using electron microscopy Falcao in the Gautier department, has shown (4) that ADP is able to stick the platelets together and to provoke a structure in some thrombasthenic platelets in five sheets between adjacent plate lets. Even if platelet populations are abnormal, if ADP acts on thrombasthenic platelets why do these not aggregate ?

I just want to consider some recent studies on thrombasthenia. First the platelet proteins we have reported that thrombasthenic platelets are frequently lacking of fibrinogen (1) but, at the present time we were unable to detect any fibrinogenopathy

In contrary with the findings of Taylor and Zucker (6) with Cronberg and Hurez (3) we

Table I

Agglutination of platelets from 4 thrombasthenic patients and 2 normal controls with various rabbit antisera (the antiserum-platelet mixtures were shaken at 23 C for 30 minutes and observed t phase contrast microscopy)

		Rabbit Antisera				Normal rabbit serum
		anti IgG	anti IgA	anti IgM	anti human plasma	
Thrombasthenia	1	±			±	
	2	+		±	+	±
	3				±	
	4				+	–
Control		+			±	±
	6	+++	++	+	++	+

Table II

Sialic acid content in platelet from 3 thrombasthenic subjects.

	μg mg protein	μg 10^8 platelets
Case 1	4 27	0 91
Case 2	5 74	2.0
Case 3	3 35	1 21
Control (7)	5 33 0 87	2 08 ± 0 26

were unable to detect a decrease in the IgM globulin on thrombasthenic platelet surface The weaker aggregation we observed (Table I) could possibly be caused by denatured proteins present in various amounts in the different antisera, since one knows that each inactivated gamma-globulin has been found also to aggregate platelets Recently Levy Toledano was able to measure both magnesium-activated ATP-ase and superprecipitation activities of thrombosthenin isolated from the platelets of one subject affected with thrombasthenia She had noticed that the superprecipitation was very weak n regard with the protein content, whereas ATP ase activity was within normal range. But, due to the fa t that this patient had a platelet iso-ant body we believe that further comment on this topic ould be valuable Recently using an anticontractile protein antiserum we have found with Booyse a decrease of the surface (S) thrombosthenin.

Levy Toledano had also measured the sialic acid content Of the three patients in whom platelet sialic acid was examined (Table II) it was found decreased in two and normal in one as expressed either in micrograms per milligram protein or in micrograms per 10^8 platelets. As the decrease in sialic acid was only found in two of the three cases, it is difficult to assess that this decrease can be considered as responsible for the defect of platelet aggregation

Now let me consider the release reaction. In contrast to other types of thrombocytopathy the platelet release is normal, at least in the presence of collagen and thrombin, whereas it is nil in presence of ADP and epinephrine (Table III) It seems, therefore that platelet factor 4 as well as nucleotide release are not necessarily related to platelet aggregation, as release is normal in thrombasthenic platelets in the presence of collagen, whereas aggregation is nil or very weak Using kaolin distilled water latex or bentonite we have found, with Cronberg, a normal, even an increased release when using washed thrombasthenic platelets (Table IV). In a previous paper (2), we have also indicated that usually the content of nucleotides in thrombasthenic platelets is normal excepted in the very rare cases in whom, as described ten years ago by the group of Gross (5) a low level of platelet ATP was found. Electron micros-

Table III

PF_4 release (in per cent) in 3 thrombasthenic patients in presence of ADP, adrenaline and collagen

Incubation time (in minutes)	0	3	5	10	0	3	5	10
Releasing agent	Thrombasthenia (3 subjects)				Control (mean of 15 determinations)			
ADP 5 μM	3-5	8-12	12-16	12-16	5	18	40	55
Adrenaline 6.2 μM	3-5	3-5	3-5	3-5	3	20	40	65
Collagen 20 μg/ml	3-5	35-60	45-90	50-50	5	45	65	70

Table IV

Platelet release in platelet suspensions (ADP as determined by aggregating activity 10^{-9} mol per 10^{9} platelets)

	N	Spontaneous 4 C	Spontaneous 37 C	Distilled [illegible] 0,095 % NaCl	Kaolin 1 g/l	Kaolin 5 g/l
Thrombasthenia	4	3.8	9.6	90		62
Control	13	4.6 ± 2.6	8.2 ± 3.6	51.6 ± 17.2	33.2 ± 19.2	49.4 ± 33.8

cope studies done in ten of our patients with Falcao (4) do not allow us to furnish any definitive conclusion on the relationship between the presence of alpha granules, lipid droplets, large mitochondria and fundamental anomaly of aggregation.

In conclusion we have many new findings on thrombasthenic platelets but we do not know what is or are the underlying anomalies responsible for the absence of platelet aggregation in this disease.

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may be considered as a non specific artefact.

The finding of a high incidence of reversible ADP-induced and absent collagen-induced platelet aggregation in a random population, which has been confirmed by other investigators (6) may be the result of aspirin. However this has been excluded as far as possible by careful questioning and repeated analysis of the urine Besides, the bleeding time showed a considerable increase following the administration of ASA (Table I)

These findings are surprising and we suggest that reversible ADP induced and absent collagen induced platelet aggregation may be considered as a physiological phenomenon in vitro

The clinical significance of these findings has still to be elucidated since two patients have been observed with a bleeding tendency a prolonged bleeding time and absent collagen-induced platelet aggregation whose prolonged bleeding times could be corrected by fresh plasma and cryoprecipitate (3)

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31 PRELIMINARY RESULTS ON TWO CASES OF ABNORMAL PLATELET AGGREGATION

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We have observed two patients with abnormal platelet behaviour similar to the one described by several authors (2 5 7)

The first patient was a 17 year old girl affected by trilogy of Fallot and with a story of recurrent epistaxis and apparently spontaneous bruising she had excessive bleeding during corrective heart surgery The second patient was a 43 year old suffering from a gastric ulcer with a story of recurrent epistaxis platelet count, one stage prothrombin time partial thromboplastin time and thromboelastographic tracings were within normal range in both patients.

In contrast platelet retention to a glass bead column (Salzman method, 6) and PF3 availability were diminished In the second patient prothrombin consumption was also decreased. Platelet aggregation was studied by the method of Born (1) in citrated platelet-rich plasma at 37°C the rate of aggregation by ADP (to a final concentration of 0.3 μM) failed to produce platelet aggregation

Collagen-induced clumping was absent in both patients as well as the second wave of aggregation brought about by noradrenaline the first wave induced by noradrenaline in contrast, was normal.

Normal platelet aggregation by ADP and thrombin was observed in both parents and in the brother of the first patient however absence of the second wave of aggregation was noted in the mother and in the brother following addition of noradrenaline

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32. ORAL CONTRACEPTION AND PLATELET AGGREGATION

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Oral contraception with conventional oestrogen-progestogen preparations results in rises of clotting factors. One of these Factor X, is involved in the intrinsic clotting system (3). When this intrinsic clotting mechanism is accelerated by physical exertion the Chandler's tube platelet aggregation time is shortened (8). Studies in women taking different types of oral contraceptive preparations, to see if their administration produces increased platelet aggregation were carried out. These were compared with a variety of parallel control groups. The studies have been continued since our first report on platelet aggregation was published in 1969 (5). The new data is described.

In the first part of the study platelet aggregation was studied by both the coagulation-induced (Chandler's tube method) and the ADP-induced optical density method. Chandler's tube aggregation was performed in women taking oral contraceptives and in two control groups, which were (1) 63 normal women and ( ) a group of 6 women in the third trimester of pregnancy. Women taking oral contraceptives were sub-divided as follows

(1) High-dose group 14 women taking conventional high-dose oral contraceptive preparations

( ) Low-dose" group 32 women taking relatively low-dose combined preparations, 14 women were on Norinyl-1 and 18 were on Ortho-Novin the latter having twice the hormone content of Norinyl-1 i.e. norethisterone ? mg and mestranol 0.1 mg to see if any changes were dose-related.

(3) Progestogen Group A 3? women taking a pure progestogen preparation Normenon (chlormadinone acetate) 0.5 mg daily and who had not taken any oral contraceptive preparation previously

(4) Progestogen Group B 15 women who had changed to Normenon from combined conventional preparations.

All the women attended at the same time of day (9.30 10.30 a.m.) and their blood was collected at mid-cycle. Chandler's tube platelet aggregation studies were performed in the progestogen groups before starting and after one three and six months of Normenon administration. Women on the relatively low-dose combined preparations Ortho-Novin and Norinyl-1 were tested at the 18 months stage only. The high-dose group had been taking oral contraceptives for an average of 26 months.

The Chandler's tube technique was modified slightly for smaller volumes of blood from the method described previously (5) as follows

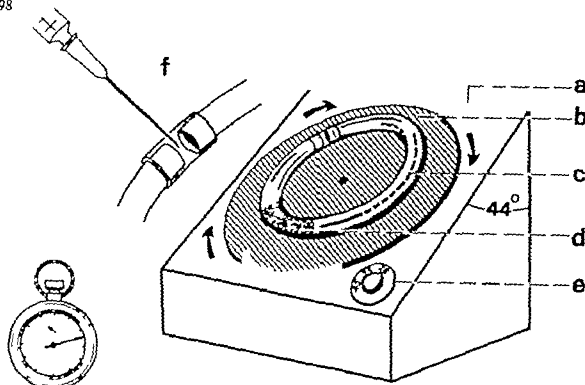


Figure 1

Home-made Chandler tube apparatus

) Wooden block (angle of 44)

b) Record-player turntable

) Polyvinyl tubing (internal diameter 3.0 m) joined t form circle.

d) Platelet-rich plasma diluted in saline/calcium mix t re

) speed control (rotate t 33 p m)

f) enlarged view of junction of plastic loop

Table I

Chandler t be ggregation times in variou p ill" groups

	T tal	Mean (min)	S.D
Female control	44	10'44	1 26
High Dose Oestrogen (Orthonovin) 36 months	11	7'27"	47"
Low Dose Oestrogen (Norin) 1 1) 36-42 months	12	7'28"	1 24
Continuous Progestogen (Normenon) 24 months	23	8'34	1 18"

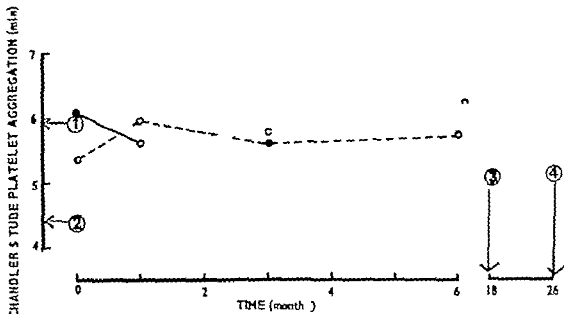


Figure 2

Platelet aggregation results (Chandler t be technique) following progestogen administration
1 Normal females
2 Third trimester of pregnancy
3. Combined preparations.
4 Combined preparations.
Progestogen groups
O——— O Not previously on ther preparations
O O Previously on ther preparations

the tube of internal diameter 0.6 cm was rotated at 16 r p m 4 ml. of 0.9 % sodium chloride and 0 2 ml of 0 25 M calcium chloride were used 2 ml. of platelet-rich plasma was added. The apparatus is seen in Figure 1

The ADP-induced optical density method of O'Brien et al. ( ) corrected for a platelet count *of 400 000 was used for platelet aggregation* studies in the 32 women starting oral contraception for the first time in the progestogen study in the women taking low-dose conven tional preparations and the normal controls. The women in the progestogen group were followed sequentially over the first six months

Results

Chandler s Tube Platelet Aggregation

Results are given in Table I The pregnant women, in their third trimester gave the shortest Chandle s tube platelet aggregation times; significantly shorter than normal and significantly shorter than any oral contraceptive group The high-dose oestrogen-progestogen group showed significantly shortened platelet aggregation Women tested after 18 months on Norinyl I or Ortho-Novin also showed significantly accelerated platelet aggregation There was no difference between the two groups. Like the changes in clotting factors, platelet aggre gation changes were not dose-dependent. The progestogen series, who had not taken an oral contraceptive (4) previously did not differ from normal initially and showed no significant change in their platelet aggregation in the first six months of study The group starting progestogen who were already taking conventional combined preparations showed significantly ac-

celerated platelet aggregation. This became normal one month after the start of chlormadinone acetate and remained normal up to the six month stage (Figure ?)

ADP-Induced Aggregation

The response to the ADP-induced optical density system showed no suggestion of change or trend either with the conventional oral contraceptives or with the progestogen series. In view of this we discontinued the use of this test in our subsequent follow-up study of the Ortho-Novin Norinyl-1 and Normenon groups

Comment

The results of women taking conventional combined "pill" preparation showed significantly accelerated platelet aggregation times with the coagulation-induced method Not only did progestogen-only oral contrapceptives not produce changes but the increased rate of platelet aggregation rapidly returned to normal when the progestogen was substituted for a combined preparation We have previously shown that coagulation factor increases result from this form of contraception and also that platelet aggregation in the Chandler's tube reflects rises and falls in clotting factors of the intrinsic system, producing acceleration and slowing respectively (5-7) A small study by Hilden et al (1) likewise found no change in ADP induced aggregation in 10 women taking an oestrogen progestogen preparation

Part 2

Follow-up Study

Follow-up coagulation and platelet aggregation studies are n progress in both combined oestrogen progestogen and progestogen (Normenon) groups Further analysis has been performed in the long-term follow-up of Ortho novin and Norinyl-1 at 36 and 4 months, and Normenon subje t up to the year stage The results are given in Figure 3 All the "pill" groups at this st ge sh w significantly acceler ated platelet aggregation in the Chandler's tube test but the Normenon (progestogen) group although significantl shorter than normal is significantly longer than the three combined

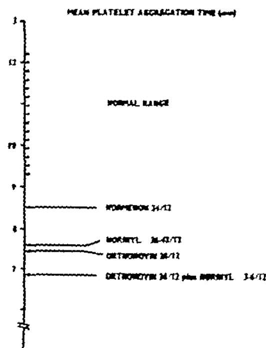


Figure 3

Platelet aggregation results after two years Normenon ompared w th other groups

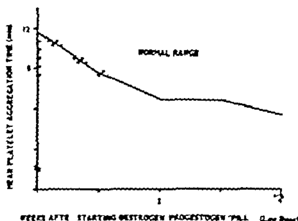


Figure 4

Mean platelet aggregation times (Chandler tube technique) in fou women studied weekly fter commencement of oestrogen progestogen pill"

oestrogen/progestogen groups.

Cases of thrombosis following conventional oral contraception have been seen by us as early as the first cycle. In our work on clotting factors we have only been able to show a marked rise in levels of Factor VII and X at the three month stage of oestrogen/progestogen administration. Although there is some individual difference in speed of response it nevertheless remained a possibility that changes in platelet aggregation might occur earlier and could be *responsible for the thrombosis risk.* Figure 4 shows results of a pilot study in four women who have volunteered to be tested at weekly intervals, after starting a combined preparation. A dramatic shortening of platelet aggregation is seen. Obviously this needs more lengthy study but the results so far are highly significant statistically (P = > 0 001).

The progestogen preparation only became available for clinical trial just over two years ago and obviously a continue follow-up will be necessary. The results after the first month of changing oestrogen/progestogen to progestogen-only contraception were dramatic. This was supported by the absence of increased aggregation over the first six months of progestogen administration in women not previously taking oral contraception. In our paper describing the first six months of the Normenon trial we emphasised that the results of clotting and platelet studies although encouraging, required a longer study to exclude gradual cumulative effects. At two years, there is some evidence of an accelerating effect on platelet aggregation with the coagulation-induced technique but the degree of acceleration is significantly less than with combined oral contraception and is greatly delayed. This appears progress towards reducing the thrombosis risk.

The platelet aggregation study raises the following questions

1. Does the lack of change in clotting factor assays mean that the Chandler's tube platelet aggregation acceleration is due to greater sensitivity of this test
2. Is the Chandler's tube measuring something else which affects the thrombin phase of platelet aggregation not covered by our coagulation studies
3. Are these platelet changes more relevant to thrombosis than the clotting changes
4. What is the explanation for the varying effects of the progestogen. Initially no change was recorded, unlike the findings with combined preparations, and the abnormal platelet aggregation with combined preparations appeared to be abolished during the first month. At two years a significant increase has been found. Is this due to compensation in the body to a long continued alteration in hormonal balance ?

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DISCUSSION

R GROSS I have two questions to Dr Caen you have assembled a lot of very valuable findings like you other people found quite different results in different kinds of thrombasthenia do you believe that these differences are caused by different diseases in a narrow sense or do you believe that it is a uniform disease and if it is a uniform disease what are your thoughts on the basic defect in this disease ? I know that it is a difficult question and you stressed at one moment that it might be impossible to answer but I believe you have some working hypotheses and I would be very eager to hear it

J CAEN Prof Gross asked me to answer a very difficult question indeed, f om the clinical point of view the two types of patients we have are equally susceptible to develop a haemorrhage from the biological point of view they are very different if both have absolutely no ADP-induced plat let aggregation, in one group (the group with subnormal platelet fibrinogen and low ATP) clot retraction is more or less normal as well as the maximal amplitude of the thromboelastogram in the other (with low platelet fibrinogen and normal ATP) clot retraction is nil. So I believe at the moment that in thrombasthenia there could be some abnormality in the relation between platelet fibrinogen and thrombosthenin, much more so in the group with low fibrinogen.

R GROSS Dr David said there was no correlation in renal diseases between platelet disturbances and the level of creatinine or urea I cannot fully accept this as in my laboratory Dr Schneider and others found that the e is a strong correlation between the level of added urea and disturbances n the citrate cycle that means in the oxydative phosphorylation

J L. DAVID I know the effect of urea at high concentration *in vitro* on the platelet function but I have not found in the literature a correlation between u ea and platelet dysfunction *in vivo*

R M HARDISTY I should just like to make two comments on Dr ten Cate s presentation first of all he told us that he found these abnormalities in 3 out of 43 normal ndividuals, but two of the three did confess to easy bruisability so t comes down to what you mean by a normal individual. Secondly I would like to ask him, in relation to the patients who had a long bleeding time corrected by cryoprecipitate and defective collagen aggregation did these patients have a factor VIII deficiency and did the cryoprecipitate correct the collagen aggregation as well as the bleeding time

J W ten CATE Concerning your first question on easy bruisability one normal individual was female and, as far as I know easy bruisability in females is a normal condition Nevertheless we found three cases of absent collagen-induced aggregation in 43 individuals and I know other investigators have the same experience Secondly we found that we could correct the prolonged bleeding time by cryoprecipitate factor VIII was completely normal in these two patients there was no coagulation defect that could be revealed by any test In one of the

three who showed a long bleeding time after aspirin we administered cryoprecipitate and, despite the fact that we could not find any abnormal coagulation factor both the bleeding time and the decreased platelet retention became normal So I think that the basic defect in these individuals is a plasma defect rather than a platelet defect. Collagen-induced aggregation, however remained abnormal after administration

H HOLMSEN I have a question to Dr Caen concerning the sialic acid determinations in the thrombasthenic platelets. Was this total sialic acid or was it that part of the platelet s sialic acid which is accessible for neuraminidase from the outside ?

J CAEN We have measured (S. Levy) the total sialic acid of the platelets treated with H_2SO 0 1 N for 60 minutes at 80°C (Warren in *J Biol. Chem. 234* 1971 1959).

J.R. O'BRIEN Like Dr ten Cate I also have difficulty in defining what is normal Among my 20 healthy apparently "normal" controls recently studied was one girl who regularly gave no response to tendon extract "collagen" She had a poor secondary response to adrenaline but other aggregation tests were normal. My version of the Salzman test was borderline low The Borchgrevink bleeding time was high normal Possibly she bruises easily Is she normal

J J SIXMA We studied a group of 40 normals, with no easy bruising among these 40 normals, five subjects showed a defective collagen (Sigma) aggregation. We had the same experience with the effect of cryoprecipitate in thrombopathia a family with this new platelet disease had a very prolonged bleeding time and absent collagen aggregation both the mother and the daughter had an operation and in both of them the bleeding time was corrected by the infusion of cryoprecipitate platelet adhesiveness was corrected too but the collagen aggregation remained absent

J HUGUES I should like to make three comments

1 I do not agree with Dr Caen that there is no collagen aggregation with thrombasthenic platelets. There is some aggregation with thrombasthenic platelets in contact with collagen but this aggregation is weaker and slower than with normal platelets. There is no aggregation with ADP

This weak aggregation of thrombasthenic platelets induced by collagen is confirmed in phase and electron microscopy

2. Just as Dr ten Cate mentioned one finds sometimes microvesicles or multivesicular bodies in the platelets. These formations are very rarely seen in normal platelets but perhaps more frequently in Glanzmann platelets. The microvesicular clusters occur with a high frequency after contact with an aggregating agent, chiefly collagen In this case they often contain dense particles which are identical in every respect to the glycogen granules located in the hyalomere

3 To Dr Sixma I must say that we never had control subjects whose platelets were not aggregated by our very highly purified collagen preparation.

J CAEN Possibly Prof Hugues is in contradiction with my results but I am not in contradiction with his in three out of the 15 patients, we reported in 1966 we have shown very slight aggregation in presence of purified collagen but usually we have no response at all with purified calf-skin collagen So it seems to me that you have possibly quite another group, if you accept the complete thrombasthenia and the incomplete one or some other mild thrombasthenia ("thromboligosthenia")

G V R. BORN In view of the difficulties with collagen suspensions and of Roka s results with procollagen, would it not be better to use procollagen from now on because it is soluble and everyone can get it in identical form ? That eliminates effects of changes in the suspension of collagen Aggregation by procollagen shows a lag phase just as with collagen

J HUGUES We always used collagen soluble in 0 4 M NaCl.

R.M. HARDISTY I should have thought that the apparent aggregation with collagen which Prof Hugues showed us, might be called adhesion which of course is normal thrombasthenic platelets do adhere to collagen, though they fail to aggregate One can obtain very similar appearances, both microscopically and on optical density curves, by mixing normal platelet-rich EDTA plasma with collagen

J HUGUES Our phase and electron microscope pictures of Glanzmann platelet aggregates induced by collagen show that these platelets not only stick to the collagen fibers but spread along these fibers and form real aggregates

R.M HARDISTY But one cannot see what is beneath that apparent aggregate those platelets may all be adhering to a mass of collagen fibres underneath.

J.R. O'BRIEN In my aggregation system, on adding tendon extract – "collagen – normally I get a delay of 30-60 seconds, then evidence of a change in shape and this is rapidly followed by aggregation (1) In thrombasthenia I usually get little or no evidence of shape change and the tracing may or may not go down a short way (possibly partial aggregation) Thereafter there is no change

P M MANNUCCI Dr ten CATE I am not sure to remember well, but when you did recently send a letter to *Lancet* was it about these patients you are referring I remember that in the journal somebody commented that the weakness of the collagen preparation you have used (which is the Sigma preparation) might be the cause of the lack of aggregation Have you tried with another collagen suspension with a human tendon extract for example ?

J W ten CATE I did not I use always a very active Sigma collagen preparation with a delay time of 24 seconds ± 4 seconds S D

P M MANNUCCI We always use the same preparation of collagen as yours, and we find it to be very active Therefore we seem to agree with you

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Question N 13

INHIBITION OF PLATELET AGGREGATION BY CHEMICALS AND DRUGS

33 INHIBITION OF PLATELET AGGREGATION BY CHEMICALS AND DRUGS

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A table of inhibitors of aggregation is shown in Table I On the left are the endogenous substances A means initial or primary aggregation and B means secondary aggregation with release The most important inhibitors are probably the endogenous ones viz. adenosine prostaglandins and fibrinogen degradation products Many of the other substances are probably of little importance because either they inhibit in much higher concentrations or they are toxic so one cannot consider using them as drugs Other inhibitors are membrane stabilizing drugs and anti-inflammatory drugs Concerning the adenosine analogues much work has shown that their inhibition is remarkably specific the most potent inhibitory substances have substitutions in position 2 of the purine ring and in the 5' position of the ribose sugar Perhaps the most interesting compound is 2-methylthio-adenosine 5'-phosphate which was synthetized by Gough and investigated by him, Maguire and Michal in Australia (2) Unlike all the other analogous substances that inhibit platelet aggregation effectively it is not a vasodilator and does not cause a fall in blood pressure in man or some other mammals I like to stress this because before this substance was tested the adenosine analogues seemed useless as drugs because of this and other unwanted effects. methylthio-adenosine 5'-phosphate is apparently specific for platelet aggregation and therefore reopens the possibility that this or another adenosine analogue could be used as a clinical drug.

Prostaglandin E (PGE) inhibits platelet aggregation (1) more strongly than any other substance so far known PGE has several pharmacological effects which are mediated by its activating action on adenyl cyclase to produce an increase in cyclic AMP There is considerable evidence that this is also how PGE_1 inhibits platelet aggregation. In my laboratory Mills and Smith (4) have shown that the inhibitory effect of PGE is very greatly potentiated by phosphodiesterase inhibitors such as

T ble I

Inhibitors of Platelet Aggregation (A) and Release (B).

Endogenous	*Exogenous*	
Adenosine	Adenosine Analogues	A
Prostaglandins	Local Anaesthetics	
Fibrinogen Degradation Products	Thiol Reagents	
	Metabolic Inhibitors	
	Guanidine Derivatives	
	Membrane Stabilizers	B
	Anti Inflammatories	

theophylline Very promising research now concerns their presentation and the demonstration of differences in the specificity of phosphodiesterase inhibitors on platelets and other tissues. This and much other evidence indicates that PGE_1 acts by increasing cyclic AMP in platelets. This is a promising observation because it opens the possibility of discovering a type of phosphodiesterase inhibitor which would greatly potentiate the effectiveness of the prostaglandin and is otherwise acceptably free of other effects.

Next, dismethylimipramine like chlorpromazine and amitryptiline and other similar drugs, do not affect the immediate aggregation produced by ADP and similar agents, but do inhibit the release reaction with increasing concentrations of dismethylimipramine the release is inhibited without affecting the first phase (3)

I do not know of any clinical efforts being made to see whether they could be potentially useful alone or in combination with something else Aspirin is an inhibitor of the second phase (release reaction) and leaves primary aggregation unaffected. Thomas (5) showed in my department that platelet adrenaline potentiates the effects of the various aggregating agents. This suggests that a drug cocktail might be effective in which an alpha blocker is included. I was once a member of a commission of pharmacologists where we said it was a terrible thing to mix drugs but for this purpose mixing drugs might be clinically advantageous.

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34 ANTI INFLAMMATORY DRUGS AND THE PREVENTION OF THROMBOSIS

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First I will consider some general properties of these aspirin-like compounds. There are many compounds that have the ability to inhibit the release reaction in platelets, for example meclofenamic acid acetylsalicylate indomethacin, ibuprofen mefenamic acid, dextropropoxyphene hydrochloride paracetamol, flufenamic etc. Clearly it is no chance coincidence that all these compounds with many different structures have both marked anti-inflammatory properties and this ability to inhibit release. It has also been shown that these compounds stabilise red cell membranes against various forms of insult (,,3). It is also probably true that they all inhibit the exposure of platelet factor 4 on the surface of the platelet (7) The speculation that the development of platelet factor 4 on the platelet surface may involve a change in mucopolysaccharide suggests a further speculation – that aspirin may influence membrane mucopolysaccharides. In all these situations it appears possible and indeed probable that aspirin is having some very generalised stabilising" effect on a membrane whether it be a platelet membrane a red cell membrane or the cells involved in inflammation Thus a detailed knowledge of the effect of aspirin on a membrane would probably teach us much about the structure and function of the membrane itself

Another remarkable property of aspirin is to cause a prolonged abnormality in the platelets long after the blood level of acetylsalicylate and even of salicylate has returned to normal. The effect can sometimes be demonstrated after taking a single dose of 150 mg of aspirin. The only other record of the persistence of an aspirin effect is that of Adams and Cobb (1). I have screened a number of anti-inflammatory compounds aspirin and a compound called Benorylate which has an easily available acetyl group caused prolonged inhibition. On the other hand, indomethacin which has no acetyl group while extremely active had a short-lived effect. Thus it was possible to speculate that the acetyl group was responsible for the persistence of the effect, although this does not explain why acetylsalicylate is effective and sodium salicylate is almost totally without effect (5).

Aspirin has now been repeatedly shown to prolong the bleeding time in normal people. Even though this prolongation is slight this indicates that aspirin has an effect on haemostasis in the normal intact human being. This effect may or may not be associated with the abnormalities detected in vitro concerning release Thus we argued that these compounds may have a therapeutic effect in preventing thrombosis. The Medical Research Council (M.R.C) of England has started a trial and in

the first instance we chose to study post-operative venous thrombosis, partly because the diagnosis using Iodine 125 labelled fibrinogen was relatively easy to establish and partly because we should be able to produce a definitive answer in a relatively short while

Basically the method consists of giving the patients potassium iodide to block the thyroid and then post-operatively injecting a small dose of Iodine-125 labelled fibrinogen. The legs are scanned with a scintillation counter every day and a localised area of unreasonably high counts indicates a "hot spot" which must signify deposition of fibrin. This test correlates very well with phlebography thus a positive Iodine 125 fibrinogen tests does indicate that a small thrombus is present in the vein Patients with a clinical thrombosis virtually always have a positive Iodine-125 test but it must be stressed that the Iodine 125 test is very frequently positive when there is no clinical evidence of thrombosis. I think we are probably getting about ten times as much thrombosis when diagnosed by this test as there is evidence of clinical disease This obviously raises important problems.

The basic plan of the protocol was to interview patients to make sure that they have not had aspirin and are not sensitive to aspirin Aspirin and a placebo were coded and each patient was given two pills, 600 mg of aspirin or placebo the day before operation and daily thereafter for five days. The Iodine-125 labelled fibrinogen was injected about one hour after the operation Thereafter the legs were scanned daily for "hot spots" The urine was collected daily from all patients and checked for the presence of aspirin When the code was opened it was essential to see that those on aspirin had a positive urine daily namely that they had taken their pills and more particularly that those who were on placebo did not have any salicylates in the urine

The official M R.C trial involves four hospitals and is still in progress Thus I can only talk of preliminary results and this only as they apply to Portsmouth. I suspect that few of these results are statistically significant and I emphasise that these are impressions rather than formally validated conclusions. So far we have studied 45 patients on aspirin and 29 % of these patients have got thrombosis as diagnosed by the Iodine-125 labelled method. Of the 4? controls 31 % have got thrombosis. Thus so far there is absolutely no evidence that aspirin has any effect in preventing this kind of thrombosis.

This study has also allowed us to measure the effect of some variables on the incidence of this thrombosis. We have been able to show that increasing age has a considerable effect in increasing the risk of this thrombosis. Dividing the patients by sex shows that males have a considerably higher risk of thrombosis. We have studied the effect of blood group and there is an apparently lower incidence of thrombosis in blood group 0 compared with the incidence in patients with other groups. Jick et al. (4) have reported that clinical thrombosis also occurs less frequently in blood group O This is of considerable importance because this is some evidence indicating that thrombosis detected by Iodine 125 labelled fibrinogen is running parallel to the clinical disease The last factor which clearly had a profound effect on the incidence of thrombosis was the type of operation. In our small series so far thoracotomy which is a major operation lasting three hours on average causes 60 % to 70 % of all patients to develop a thrombosis. On the other hand, a hysterectomy lasting less than one hour causes less than 20 % of patients to get thrombosis. All these factors have been studied in isolation and even taking into account the other factors there is still a suggestion that they remain independently valid.

I have published a suggestion that when a "hot spot" is detected with an unreasonably high count, it is the excess counts above what might normally be expected which are of importance in indicating the thrombus. This implies that a more or less normal amount of blood is passing either through this vein or through the collateral circulation in spite of the presence of a thrombus. Studying these excess counts day after day enables one to show that

in some patients a thrombus apparently remains stationary. This would indicate a single episode possibly during the operation or immediately after which is not then altered. On other occasions there is evidence of one or more thrombi increasing rapidly over a period of one or two days, indicating presumably a generalised tendency to lay down further fibrinogen. Is this a hyper-coagulable state? In other patients two or more thrombi have all decreased in size presumably indicating generalised lysis. Equally I have recorded in a number of patients a number of thrombi, some of which have decreased in size over the days and others have increased. I think this indicates the importance of local events and corresponds well with the histological picture of layers of thrombus deposition interspersed possibly with episodes of lysis. These findings encourage me to develop a method for more or less continuous monitoring of the legs so that precise details of the evolution of these thrombi can be closely watched (6).

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35 THE EFFECT OF ASPIRIN ON THE BLEEDING TIME

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The effect of aspirin on the bleeding time depends partly on the technique being used (Figure 1) Because of the inconsistent results of the Duke technique (2) after aspirin it has been decided not to use this method Considering Ivy's (3) and Borchgrevink's technique (1) *standardized according to Mielke (4) we found* the latter the most sensitive With this technique a significant increase of the bleeding time could be demonstrated in 40 volunteers after intake of 20 mg of aspirin per kg body weight. Before aspirin the mean bleeding time was 4.5 *minutes with a standard deviation of 2 minutes.* 24 Hours after intake of aspirin the mean *bleeding time increased* to 6 minutes with a standard deviation of 3 minutes

Concurrent investigation of the platelet functions showed a significant decrease of the *platelet retention in the glass bead column test* and distinct alteration of the platelet aggregation patterns, e g. reversible ADP-induced and poor and reversible or even absent collagen-induced platelet aggregation.

It is tempting to conclude that the observed increase of the bleeding time *might be due to* impairment of the platelet function which could be demonstrated in vitro However there is some doubt when we consider the following

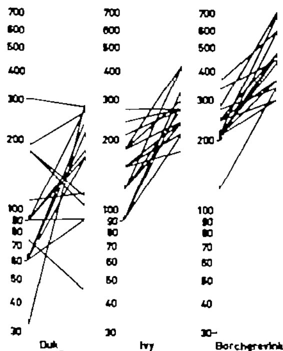


Figure 1

The left axis of each column represents the bleeding time in seconds before administration of aspirin and the right axis represents the bleeding time 24 hours after aspirin ingestion.

facts. Among the volunteers, three individuals showed after aspirin an extreme prolongation of the bleeding time to respectively 18 16 and 30 minutes. One of these volunteers received a small dose of cryoprecipitate which induced a correction of both the bleeding time and the abnormal platelet retention in the glass bead column (Table I-a) This correction of the aspirin induced prolongation of the bleeding time took place despite the fact that all other coagulation studies, including factor VIII did not reveal abnormal results.

Two patients (C 10 and He-II 2) with von Willebrand's disease showed a decreased platelet retention in the glass bead column and a slight decrease of the factor VIII activity of respec tively 59 and 35 % Following the administration of aspirin the bleeding time showed a distinct prolongation which could be corrected by either fresh plasma or cryoprecipitate (Table I-b,c) The decreased platelet retention showed a correction at the same time

A third patient (Ve 1) being treated with oral anticoagulants took 20 g of aspirin. She also showed a marked prolongation of the bleeding time which showed a partial correction after infusion of P.P.S.B (Table I-d).

These findings clearly indicate that the combination of a plasma factor defect and a platelet defect in these patients induced by aspirin, results in gross prolongation of the bleeding time This is even more demonstrated by the reduction of the bleeding time following the correction of the plasma factor defect.

Furthermore it may be concluded that the glass bead column test estimates rather a plasma factor than a platelet factor because of the correction of decreased platelet retention values after infusion of plasma or cryoprecipitate This plasma factor may be identical to the anti-bleeding factor which is known to be absent in von Willebrand's disease

This assumption results in the hypothesis that the distribution of the bleeding time after intake of aspirin might correspond with the distribution of this "anti-bleeding factor

T ble I

Correction of the ASA-prolonged bleeding time after inf sion of fresh plasma and plasma preparations.

(a) Volunteer A.

Method	Before ASA	After ASA	60 min after 4 U of cryoprecipitate
Bleeding time (min)	4,5	18	9
Glass bead retention (%)	40	26	53

(b) Patient C. 10.

Method	Before ASA	After ASA	60 min after 8 U of cryoprecipitate
Bleeding time (min)	6,5	30	5
Glass bead retention (%)	36	35	55

(c) Patient He-II-2

Method	Before ASA	After ASA	60 min after 400 ml of fresh plasma
Bleeding time (min)	5	16	6
Glass bead retention (%)	49	41	71

(d) Patient Ve-1

Method	Before PPSB	After PPSB
Bleeding time (min)	20	14
Prothrombin time (sec)	135	17

One U f cryoprecipitate is prepared from 250 ml of fresh plasma.

PPSB was administered an amount equivalent to 1500 ml of plasma.

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36 EFFECT OF ASPIRIN ON PLATELET AGGREGATION AND BLEEDING TIME IN HAEMOPHILIA AND VON WILLEBRAND S DISEASE

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It is well known that acetylsalicilic acid (ASA) can increase the haemorrhagic diathesis of haemophiliac patients (1) While small doses of aspirin (0.5-1 gr) slightly prolong the bleeding time of healthy people its effect in some haemophiliac and von Willebrand's patients is much more remarkable (2) Disappearance of second wave of ADP and adrenaline-induced aggregation are usually observed with ASA in vitro (3). In vivo the effect of a single oral dose of aspirin (1 gr) on platelet aggregation is still evident after 3 days (Figure 1)

We have correlated bleeding time and platelet aggregation in haemophiliac and von Willebrand s patients to observe whether the greater increase of the bleeding time in these patients may be related to a behaviour of the platelet aggregation different than in normal controls.

Seven normal subjects, 7 patients affected by haemophilia A 3 affected by haemophilia B and affected by on Willebrand disease received 1 gm aspirin per os in the morning in fasting state Bleeding time (Duke), ADP ($6x10^{-4}M$) and adrenaline ($9\ 2x10^{-4}M$)* induced platelet aggregation were performed before drug ingestion hours and 3 days after

Platelet aggregation at 37°C with contin ous stirring was studied by a turbidimetric method using the platelet aggregation meter (EEL 169) the changes in optical transmittance were graphically recorded

After hours the bleeding time was much more prolonged in haemophiliac and von Willebrand's patients than in normal controls (Figure ?) no significant differences were present after 3 days

Several patients presented minor haemorrhagic complications rebleeding about 1 hour after the test (4 patients) little haematoma of the ear lobe (1 patient) haematoma of fore-arm (1 patient) and large ecchimosis at the point of withdrawal (patients) No haemorrhagic complications were observed in the controls

Platelet aggregation was studied mainly to observe the presence of a) secondary aggregation b) disaggregation more than 70 per cent of the initial aggregation

Concerning ADP-induced platelet aggregation the behaviour of the two parameters after ASA administration was similar in the patient s and in the control's group all subjects showed disaggregation greater than 70 %

* final concentration

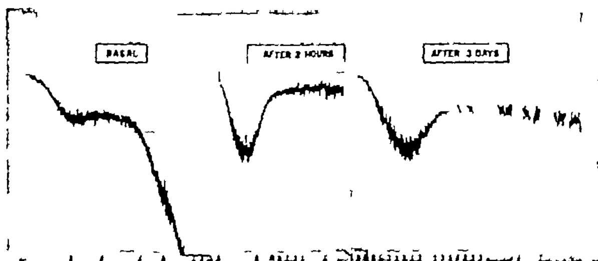


Figure 1

Effect of single dose of ASA (1 gr per os) on adrenaline (final concentration 9.2x10[illegible] M) induced platelet aggregation in a normal subject.

TWO HOURS AFTER ASPIRIN 1gr

BASAL

BLEEDING TIME (DUKE) MINUTES

20

15

10

5

4

3

2

1

NORMAL SUBJECTS

HAEMOPHILIA A

HAEMOPHILIA B

VON WILLEBRAND'S DISEASE

Figure 2

Bleeding time before and two hours after ASA (1 gr per os)

DISCUSSION

M VERSTRAETE I have a question to Dr O Brien In one tenth of your patients with an excess count of radioactive fibrinogen there was clinical evidence of phlebothrombosis. Did you or did someone perform venography in the 90 per cent of patients who did not have evidence of phlebothrombosis but who had an excess of radioactive count ?

J R. O'BRIEN We have done no phlebography in Portsmouth or indeed in this trial. However Negus et al. (1968) have shown a high degree of correlation between phlebography and the Iodine-125 method In almost every case where he found a hot spot there was clear-cut evidence by phlebography of thrombosis.

G V.R. BORN I should like to ask Dr O Brien the obvious question why venous thrombosis and not arterial thrombosis ? It is platelets we are interested in and the most definite out come of this trial seems to be to confirm that platelets are not involved in venous thrombosis, not even in its initiation Why did you not study some arterial situation

J R. O'BRIEN I think that venous thrombosis was chosen because it was possible to carry out this study in a reasonable time and for a reasonable expenditure of money Clearly aspirin might have worked in venous thrombosis (indeed the negative has not yet been proved) I imagine that platelets must play some part in venous thrombosis even if it is only to trigger off the clotting system. I agree with Professor Born that platelets probably play a greater part in arterial thrombosis and I think it of the greatest importance that some kind of trial of aspirin or one of the anti-inflammatory drugs is organised in arterial thrombosis Ideally such a trial requires that half the patients studied should never take aspirin We have evidence that the eating of aspirin is extremely widespread and it is probably impossible to have in real life a pure" control group who never eat aspirin This is clearly a major snag to the design of a suitable experiment The other problem in organising an investigation for the prevention of arterial disease is the relative infrequency of arterial thrombotic events Even the study of secondary prevention of myocardial infarction probably requires a close study of 10,000 patients for five years Thus I entirely agree with Professor Born that such a study should be undertaken but its organisational problems and cost will be great

A.S DOUGLAS If I could follow on from Dr O'Brien I conclude from his account that the use of [125] I fibrinogen was in order to have an objective measure of the incidence of thrombosis. On the question of a population taking unprescribed aspirin, I do not think this matters too much because the design is testing the intention to treat one group of patients with aspirin the other group not being so treated. The ingestion of aspirin by those not intended to be so treated does not necessarily spoil the design provided the incidence of thrombosis is referred back to the intention to treat.

G V R. BORN Dr Evans and Dr Mustard have shown that aspirin diminishes platelet deposition after arterial operations and in arterial protheses which remain patent for longer than in the absence of aspirin

Table I

In vitr distribution f [1] C labelled aggregation inhibitors in plasma and in sediment afte 20 min. incubation in platelet-rich plasma.

	ASA carboxyl-labelled	ASA acetyl-labelled	Ra433	Ra233	Persantin	Intensain (carbochromen)
CpM/Vol. Plasma / CpM/Vol.Sed.	1 1.07	1 1.04	1 1.5	1 1.7	1 0.8	1 29.4

K. BREDDIN We have just finished a pilot study in the surgical department in Frankfurt where patients received or did not receive aspirin in a dose of 1.5 gr per day (450 patients on each side) the results in the two groups were 17 thrombo-embolic episodes in the non-treated and six thromboembolic episodes with no deleterious pulmonary embolism in the aspirin treated group From the findings of this one year trial which is somewhat in contradiction with the findings we have just heard, we decided it would be worthwhile to do a controlled double blind study on the effect of aspirin Table I shows what we found investigating radioactively labelled aggregation inhibitors and their binding to platelets. There was no significant binding of either the carboxyl-labelled or acetyl-labelled acetylsalicylic acid (ASA) we also found no binding of one of the pyrimido-pyrimidine compounds to the platelets but we found a very high binding of carbochromen which is a relatively weak aggregation inhibitor but which after ten minutes of incubation time is concentrated thirtyfold in the platelets This binding of carbochromen is dependent on sodium ions and can be inhibited by ouabain The effect of aspirin lasts in different persons from two to ten days, usually between three and four days (Figure 1). Thus, we wondered if this had something to do with platelet turn-over We should expect that if the aggregation inhibiting effect of ASA had something to do with its binding on the platelets, there should be a difference in platelet turn over which is not the case It is interesting that the effective time of inhibition by aspirin is quite constant for the same individual. We have tried to find out whether in vitro there is a binding of the carboxyl label of salicylic acid on proteins (Figure ?) There is actually a strong binding to albumin If this experiment is repeated with ^{14}C acetyl labelled ASA there is only a very small binding of ^{14}C label to

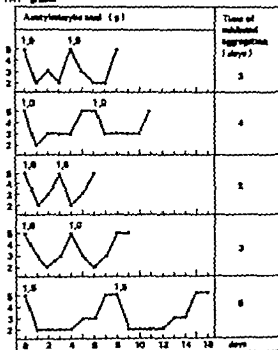
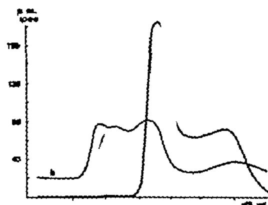


Figure 1

After ingestion f 1 or 1.5 g of acetylsalycilic acid the aggregation inhibiting ffect lasts for 2-6 day The duration of effect is fairly constant for each individual



F gure 2

After 30 mm of incubation with C carboxyl-labelled ASA, part of the [1] C label was bound to the albumin fraction (curve) Separation of plasma on Sephadex G 200 (curve b)



Figure 3

Separation of plasma sampled 2 hours after oral intake of C acetyl labelled ASA, on Sephadex G 200 (curve b) Part f the radioactivity was found in the globulin fraction (curve a)

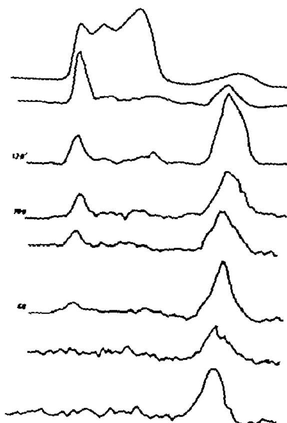


Figure 4

Sixty min after intake of C acetyl labelled ASA small amount of radioactive material was bound to the globulin fraction (plasma separated on Sephadex G 200 upper curve) During the follo ng hours the globulin bound radioactivity rose. Platelet aggregation was inhibited already in the 40 min sample.

globulins After oral intake of carboxyl C labelled ASA most of the activity was found in the urine within 24 hours. If plasma of volunteers was separated by G-200 gel filtration a few hours after the oral intake of [4]C carboxyl labelled ASA a strong albumin binding was observed. Twenty four hours later no activity was detectable in the plasma.

After oral intake [4]C acetyl labelled ASA was very slowly excreted globulin bound activity was found shortly after ingestion (Figure 3). Figure 4 shows the fractionation of plasma samples taken at 20 min intervals after ingestion of acetyl [1] C labelled ASA. An inhibitory effect on platelet aggregation was not found after 20 min but was distinctly present in the 40 min sample The globulin binding was detectable after 60 min. It increased during the following hours and remained constant for the following 8-14 days. The labelled material is actually a very low density lipoprotein it is not fibrinogen.

G V R. BORN I was surprised by these results because the label persists for ten to fourteen

days. This indicates a protein with a remarkably slow turn-over

J.R. O BRIEN I gather these experiments were done with serum and serum contains some break-down products of platelets. Have you done any experiments with plasma ?

K. BREDDIN It is the same with plasma and serum we prefer serum because fibrinogen sometimes occludes the columns. Of course we tried to detect some of the label on the platelets, but there was no rise in platelet label at any time. So we find no binding of either the carboxyl group or the acetyl group to the platelets. As far as the turnover is concerned the label diminishes from day to day but it is detectable up to the fourteenth day the half life being 10-12 days in the persons we have tested.

S. CRONBERG In our experiments with washed platelets, we did never see any second wave of aggregation of platelets harvested after the intake of acetylsalicylic acid on the contrary washed platelet suspensions prepared before the intake of acetylsalicylic acid showed a second wave of aggregation after addition of ADP or adrenaline in the presence of platelet poor plasma thus plasma could be also taken from individuals after the intake of acetylsalicylic acid thus the action of aspirin was on the platelets, and has nothing to do with the plasmatic factor

J R. O BRIEN I would like to support Dr Cronberg s observation, namely that plasma from people who have eaten aspirin two days before has no effect in inhibiting the release reaction

J CAEN I was surprised by the results of Dr Breddin who did not find any difference between the binding of the acetyl and carboxyl groups of acetylsalicylic acid to platelets to my knowledge Marcus et al. found a considerable difference between the binding of both groups to platelets.

K. BREDDIN Our results were confirmed lately by Al-Mondhiry Marcus and Spaet, who also found no binding of the acetyl group of ASA to platelets.

I agree with Dr Cronberg s findings on the release reaction. The problem is however what the release reaction actually means. Our test system is completely different from the tests which rely on ADP-induced aggregation. We do not add any aggregating substance In our test system the aggregation inhibition still is present two days after ingestion of aspirin Here we come to the very difficult question what the different test systems actually measure and of what use they are for clinical investigations.

J CAEN I have a question to Dr ten Cate or any other member of this symposium has anybody seen what happens in the plasma of patients treated with aspirin in regard to the degradation of ADP ? Do you believe that the result of plasma or cryoprecipitate transfusions could be due to some effect in this regard ?

J W ten CATE I do not think this is possible because the volume of the administered cryoprecipitate is small, it is about 50 ml secondly the response of the platelets after infusion was not altered compared to the aggregation patterns before infusion of cryoprecipitate or plasma this means that in the aspirin group the reversible ADP and absent collagen-induced aggregation remained unaltered by the infusion the correction by cryoprecipitate of the aspirin-prolonged bleeding in similar cases has in the meantime been confirmed by dr Veltkamp who is working in the Department of Haemostasis at Leiden (Head Prof E.A Loeliger)

K BREDDIN We have lately been considering the possibility that aspirin has a direct effect on the platelets, which we measure in our in vitro test systems during the first hours after aspirin intake and a second effect on the plasma which lasts much longer Is this a way to explain our partially conflicting results ?

J R O BRIEN I am most interested in Dr ten

Cate s finding that the transfusion of cryoglobulin into these patients caused a shortening of the bleeding time Have you transfused cryoglobulin into normal people to see if it shortens the bleeding time ? You will remember that the transfusion of almost any fluid raises factor VIII in many people presumably as a result of stress.

J W ten CATE I did not administer cryoprecipitate to normals, but I did so to haemophiliacs and I studied the bleeding time and the glass bead retention values there was *no significant change I administered cryo*precipitate to two patients with uraemia, who are known to have prolonged bleeding time and abnormal glass bead retention I found no influence of cryoprecipitate

R GROSS I have a question to Dr Praga as you know von Willebrand's disease is a vascular disturbance combined with plasma defects or in some cases with platelet defects can you distinguish with the aspirin test between these two kinds or types of von Willebrand's syndrome

C PRAGA Platelet aggregation was always normal in all Von Willebrand's patients I have seen so far In the two cases here presented, the platelet aggregation after ASA was exactly the same as in normal people So I cannot answer your question.

J W ten CATE I think this is very simple aspirin induces a platelet defect a platelet defect plus a plasma deficiency induces a long bleeding time this has been demonstrated already 20 years ago

J VERMYLEN Dr ten Cate have you seen patients in whom you were convinced that aspirin was the cause of bleeding, for instance women with menorrhagia ? Did interruption of aspirin ingestion result in reduced bleeding ?

J W ten CATE I studied a family of 35 patients from June until August this year who attended our laboratory for bleeding tendency very similar to von Willebrand's bleeding tendency all the patients had proven use of aspirin this was demonstrated by abnormal platelet aggregation which was reversible after discarding the use of aspirin.

J VERMYLEN Do you think there is a certain predisposition in these persons to bleed after ingestion of aspirin ?

J W ten CATE We are going to investigate this it might be possible

B. DIPYRIDAMOLE AND RELATED SUBSTANCES

37 A.S. DOUGLAS Action of Pyrimido-pyrimidine Compounds on Platelet Behaviour *in vitro*.

38 J.J. SIXMA and A. TRIESNIGC The Inhibition of the Function of Human Blood Platelets *in vitro* by VK 744

39 H. REUTER The Effect of the Pyrimido-pyramidine RA 233 on Function and Metabolism *of Human Platelets*.

DISCUSSION

H. SCHRÖTER K. BREDDIN

37 INTRODUCTION
ACTION OF PYRIMIDO-PYRIMIDINE COMPOUNDS ON PLATELET BEHAVIOUR IN VITRO

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This is an introduction to this section on the action of pyrimido-pyrimidine compounds on platelets The work described has been published previously with associates in the University Department of Medicine Royal Infirmary Glasgow (Drs A.A. Hassanein, A.G G Turpie G P McNicol C D. Forbes)

The major contribution of platelets to the formation of intravascular thrombi has stimulated the search for agents capable of inhibiting platelet aggregation It has been previously established that dipyridamole (Persantin R.A.8) can inhibit platelet aggregation (6 7).

With this compound however it is very difficult to achieve adequate concentrations "in vivo" without unacceptable side-effects. Two synthetic dipyridamole analogues (R.A.433 and R A 233) have been studied in vitro and shown to interfere with platelet function in a number of test systems both compounds have been found, in vitro to be more powerful inhibitors of platelet aggregation and adhesiveness than dipyridamole This paper summarises the findings on R.A 433 and R.A.233 as previously published (8 11) Studies on R A.433 have also been reported by other investigators (5)

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Chemistry

R.A 433 is 2, 4 6 – trimorpholino-pyrimido (5 4-d)-pyrimidine

R.A. 233 is 2,6-bis(diethanolamino-4-piperidino-pyrimido (5 4-d) pyrimidine.

These compounds are insoluble in water but soluble in dilute acid.

Materials

R.A.233 (Boehringer Ingelheim). Stock solution 10^{-4}M R.A.233 in 0.025 N hydrochloric acid stores at 4 C Dilutions referred to in text were made in normal saline

R.A.433 (Boehringer Ingelheim). Stock solution 10^{-4}M R.A. 433 in 0 025 N hydrochloric acid stored at 4 C Dilutions referred to in text were made in normal saline.

Adenosine 5-diphosphate ADP (Sigma Chemical Company St. Louis) Stock solution 100 µg/ml ADP in barbitone/saline buffer pH 7.2 stored at -20°C. Dilutions were made in barbitone/saline buffer

Tubing. Chandler tube experiments. Transparent vinyl tubing (MT/13 Portland Plastics, Kent) and plastic adaptors (10M/634 Portex).

Glass bead columns. Transparent vinyl tubing (MT/13 Portland Plastics, Kent) translucent silicone tubing (Esco Rubber Ltd., London)

Ballotini glass beads 0.57 mm diameter
Calcium chloride M/4 M/40.
Kaolin 5 per cent kaolin in imidazole buffered saline pH 7 2.
Russel viper venom (Stypven Burroughs Wellcome Co).

Citrated blood was collected by clean vene puncture in plastic syringes using 21-gauge needles nine volumes of blood being mixed with one volume of 3.8 per cent trisodium citrate in siliconized graduated centrifuge tubes maintained at room temperature Platelet rich plasma (PRP) was obtained by centrifugation of citrated whole blood at 600 g for 5 minutes at room temperature Siliconized glassware was used throughout (Siliclad Clay Adams Inc New Jersey)

Methods

Calcium-induced platelet aggregation – Chandler tube technique

The method used was that described by Chandler (1958) as modified by Cunningham et al. (2) ?9 ml. normal saline 1 ml. PRP and 0 04 ml. of R.A.?33 or R.A 433 to a final concentration of 5×10^{-4} M were incubated in a vinyl plastic loop for 5 minutes at 37°C After incubation recalcification of the mixture was carried out with 0 1 ml M/4 calcium chloride In the control observations the drug solvent was substituted for the solutions of R.A.433 or R.A.233 The recalcification to platelet aggregation time was measured the end point of aggregation was regarded as the production of the "snow-storm" effect of platelet aggregates at the leading edge of the plasma.

Turbidimetric method.

Born s method (1) was modified as described by Hassanein (10) to assess the calcium induced platelet aggregation Two ml of PRP diluted 1 4 with normal saline was incubated with 0.02 ml of R.A.233 or R.A 433 (final concentration of 5×10^{-4} M) at room temperature for 5 minutes. From this incubation mixture 1 9 ml. was transferred to the perspex cuvette and the mixture stirred at room temperature in an EEL titrator connected to a galvanometer (EEL unigalvo type 20) for 30 seconds the optical density arbitrarily set at 0 600 and therafter the mixture recalcified with a mixture of 0 05 ml. M/4 calcium chloride and 0.05 ml N saline. Following recalcification optical density readings were taken at 5 second intervals until coagulation occurred In the control experiments solvent was used in lieu of the drug solution. On the addition of calcium chloride to stirred diluted control PRP an initial fall in optical density occurred due to dilution there followed a lag phase in which no appreciable change in optical density was observed, then a slight increase in optical density followed by a fall due to platelet aggregation finally clot formation occurred. The results obtained using this system are expressed as follows

Aggregation time time taken for the initial fall in optical density due to platelet aggregation occurring.

Duration of platelet aggregation time interval between the beginning of the initial fall in optical density due to platelet aggregation and coagulation

Optical density fall magnitude of the fall in optical density occurring as a result of platelet aggregation

Clotting time time interval between recalcification and fibrin formation

ADP induced platelet aggregation.

The turbidimetric method of Born (1) was used, details of the apparatus being given above 0.02 ml of each drug solution or of solvent control was incubated with ? ml of PRP at room temperature for 5 minutes (final concentrations of R.A 233 and R A 433 5×10^{-4} M and 2.5×10^{-4} M) 1 9 ml of the mixture was stirred for 30 seconds in the perspex cuvette on an EEL titrator connected to a galvanometer and the optical density arbitrarily set at 0.600. ADP in a final concentration of 0.25 μg/ml was added and the optical density recorded at 30 second intervals over 10 minutes. The experiments were conducted at room temperature none were carried out at 37°C Indices of platelet aggregation and disaggregation are expressed as follows

30-60 platelet aggregation fall in optical

density between 30 and 60 seconds after the addition of ADP

Maximal aggregation difference between the lowest recorded optical density reading and the constant arbitrary base-line of 0 600.

Percentage disaggregation ratio between the increase in optical density occurring 5 minutes after the point of maximum aggregation and the maximum platelet aggregation.

Platelet adhesiveness.

The glass bead column technique of Hellem (12) as modified by Hirsh et al. (13) was used. 4 ml of citrated whole blood, which had been kept at room temperature for 30 minutes, was incubated for 10 minutes at room temperature with R.A.233 or R.A.433 (final concentrations of 10^{-4} M or solvent control) inversion was used in order to obtain mixing. After incubation and further mixing percentage platelet adhesiveness retention in glass bead column was determined using glass bead columns

Platelet factor 3 availability

A modification of the technique of Spaet and Cintron (14) was used 0 9 ml of PRP was incubated for 10 minutes at 37°C with R.A.233 or R.A.433 in final concentrations of 5×10^{-4} M and 10^{-4} M, or with corresponding solvent control. 0.1 ml 5% kaolin in imidazole buffer was added and incubation continued for a further 30 minutes at 37°C after incubation the kaolin was resuspended. 0 1 ml was added to a mixture of 0 1 ml M/40 calcium chloride and 0 1 ml Russel viper venom (10 μg./ml.) at 37°C and the clotting times determined in duplicate To determine the effect of the drugs on plasma coagulation factors in the concentrations used in the experiments, R.A.233 and R A 433 together with solvent controls were incubated with platelet poor plasma (PPP) and the same experiments repeated.

Whole blood clot retraction.

A modification of the method described by Dacie (3) was used 5 ml of whole blood was mixed in siliconized centrifuge tubes containing copper wire spirals with 0.25 ml of R.A.233 or R.A.433 (final concentrations of 5×10^{-4} M), or solvent control The mixtures were allowed to clot and the tubes incubated in a water bath at 37°C for 2 hours. The copper wires with adherent clots were then gently removed from the tubes. The remaining serum volume was recorded after centrifugation at 1000 g for 15 minutes and the percentage clot retraction calculated compared with a theoretical maximum obtained from the haematocrit reading.

Plasma clot retraction with added ADP

2 ml citrated PRP was added to siliconized centrifuge tubes containing cooper wire spirals and with 0. ml of R.A.233 or R.A.433 (final concentration 5×10^{-4} M) or solvent control, and the mixtures incubated at 37°C for 10 minutes. 0.1 ml ADP (final concentration 5 μg/ml) was added and incubation continued for a further 5 minutes. Recalcification with 0.2 ml M/4 calcium chloride was carried out and the plasma clots incubated for 2 hours at 37°C in a water bath. The wire loops and adherent clots were then removed, the remaining serum measured clot retraction was expressed as a percentage of the total volume Experiments without ADP were performed and the results compared

Platelet counts.

These were performed using formal citrate as the diluting fluid (3) a conventional light microscope was used.

Results

These are only given in summary and the original papers (8 11) should be consulted for a detailed account of the results.

Calcium induced platelet aggregation. Chandler tube technique

Seven experiments were carried out using R.A 233 and R.A.433 in final concentrations of 5×10^{-4} M compared with solvent controls. Both drugs produced a significant prolongation of aggregation time (i.e. appearance of the snow-storm effect)

Turbidimetric method.

Seven observations were made with each drug and the same number of control studies. R.A.233 significantly prolonged the aggregation time whereas with R.A.433 while there was a similar trend it was not significant at con-

ventional levels. The duration of platelet aggregation and optical density fall were diminished at levels of statistical significance by both drugs. Neither drug influenced the coagulation time significantly

ADP induced platelet aggregation.

Seven experiments were carried out using R.A.233 and R.A.433 in concentrations of 5×10^{-4}M and 5×10^{-4}M compared with solvent controls. Both drugs produced significant inhibition of both 30-60 second aggregation and maximal platelet aggregation. Neither drug influenced platelet disaggregation in the test systems using normal platelet rich plasma

Platelet disaggregation.

A defect in platelet disaggregation using ADP as an aggregating agent in the turbidimetric system of Born has been described in uraemic patients (10) The effect of R.A 233 on ADP induced platelet aggregation in 7 patients with chronic renal failure was studied both of these patients exhibited abnormal platelet disaggregation R.A 233 reversed platelet disaggregation to the expected normal values.

Five out of 11 patients with peripheral vascular disease all of whom were on anticoagulants, showed diminished percentage platelet disaggregation when studied in the turbidimetric system. R.A.233 in a concentration of 2.5×10^{-4}M significantly increased the percentage platelet disaggregation in each case In the remaining six patients with normal platelet disaggregation, R.A 233 had no significant effect

Platelet adhesiveness

Seven experiments were conducted with R A. 33 and R A 433 in final concentrations of 10^{-4}M and the results compared with those obtained with solvent controls Both drugs reduced platelet adhesiveness by a significant amount. R.A. 33 was more potent than R.A 433

Platelet factor 3 availability

R.A.233 and R.A.433 significantly reduced platelet factor 3 availability at concentrations of 5×10^{-4}M and 10^{-4}M Neither drug affected the coagulation times when PPP was used indicating that the drugs had no inhibitory effect on plasma coagulation factors.

Clot retraction.

Whole blood clot retraction was diminished both by R.A.233 and R.A.433 the clot retraction of recalcified platelet rich plasma was also significantly diminished by both drugs. The addition of ADP prior to recalcification partially corrected the inhibition of retraction produced by R.A 233 but the ADP had no significant effect on the inhibition produced by R.A 433.

Discussion

Interest in the pyrimido-pyrimidine compounds has arisen because of the possible therapeutic role in arterial and venous thrombotic vascular occlusions There is considerable evidence that adherence of platelets to one another and to the vessel wall at sites of endothelial injury is an early stage in thrombus formation The search for drugs to reduce platelet adhesiveness is therefore a logical step to the therapeutic approach. The pyrimido-pyrimidine derivatives, dipyridamole R A 433 and R A 233 (5,6 7,8 9 11) have been shown to reduce platelet adhesiveness/aggregation in vitro It is not justifiable to extrapolate this evidence to an immediate conclusion that these compounds are necessarily effective in vivo they can have differing actions in the injured artery model in experimental animals dipyridamole inhibites thrombus formation whereas R.A.433 was ineffective (5) in a different animal model (4) R.A.433 inhibited thrombogenesis in the rat in an arteriovenous teflon microshunt technique

Although R A 433 and R.A 233 are more powerful in vitro than R A.8 this does not mean that necessarily they have a more significant role in man. Many other issues require to be explored including toxicity and drug metabolism.

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38 THE INHIBITION OF THE FUNCTION OF HUMAN BLOOD PLATELETS IN VITRO BY VK 744

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The inhibition of platelet function by VK 744 a dipyridamole congener was studied.

VK 744 inhibits collagen-induced aggregation at a concentration of 25 μM both at 37°C and at 20°C. Preincubation at 37°C up to 30 minutes enhances the effect. ADP-induced aggregation (10 μM ADP) was inhibited by 50 μM VK 744 at 37°C and at 20 C. Adrenaline induced aggregation was inhibited too. This inhibition showed itself by reversible aggregation in the presence of 50 μM VK 744. VK 744 produces active disaggregation when added after aggregates induced by ADP has been formed.

The glass bead column retention test (3) and clot retraction (4) were inhibited at a concentration of 1 mM. Platelet factor 3 availability as well as serotonin uptake were inhibited with a ED 50 (effective dose that gives 50 % of the effect) of 6 μM and 0.2 mM respectively. Comparison with the inhibition by RA 233 showed that these effects were both mediated by two separate pharmacologic receptors. Investigations on the release of C^{14}-serotonin during collagen-induced aggregation showed that VK 744 is a primary aggregation inhibitor.

From the log-dose response relation during ADP-induced aggregation in the presence or absence of VK 744 (at 20°C) it could be concluded that VK 744 is a competitive inhibitor.

Investigations by Born et al. ( ) have shown that inhibitors of ADP-induced aggregation of the adenosine type show a very strong relationship to adenosine itself. This suggests that the inhibition by VK 744 is not a real competitive inhibition but a so-called functional inhibition (1). These results will be published in extenso elsewhere.

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39 THE EFFECT OF THE PYRIMIDOPYRIMIDINE RA 233 ON FUNCTION AND METABOLISM OF HUMAN PLATELETS*

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2 5 Bis(diethanolamino) 4-piperidino-pyrimido-(5 4-d)pyrimidine (RA 233) in 10^{-4} to 10^{-4}M concentrations inhibits adhesion and aggregation of human platelets in vitro After intravenous administration of a single dose of either 100 or 150 mg RA 233 the adhesion of platelets to glass beads (measured by the method of Hellem) is also significantly reduced. As can be seen from Table I adhesion of the platelets was decreased in citrated blood 10 minutes after injection. In 2 persons a significantly decreased adhesion could be observed up to 240 minutes after administration Blood levels of RA 233 after intravenous infusion decrease rapidly and about 10 minutes after injection concentrations of 1γ/ml are found. RA 233 in this concentration does not influence platelet function in vitro

Furthermo e it is conspicuous that a higher dose of RA 233 has a rather weaker effect than the lower one

The spreading capacity of the platelets was inhibited only in one of 10 persons after administration of 100 mg RA 233 Aggregation by ADP adrenaline and collagen was not significantly affected by RA 233 Intravenous administration of daily doses of 3 x 100 mg RA 233 for 10 days is well tolerated according to our experience As shown in Figure 1 treatment with RA 233 results in a distinct decrease of adhesion. From laboratory controls there is no indication that RA 233 may influence the functions of liver and kidney The data given in Figure 1 have been obtained from a patient who was admitted to the hospital because of disturbances of peripheral blood supply The initial therapy with Tebonin was stopped one week after admission and 3 days later RA 233 was applied for 10 days After a therapy free interval of 8 days RA 233 was given again for 6 days. During this time the patient s peripheral blood supply improved very well the adhesion values being 11 23 %

In vitro RA 233 is able to disaggregate platelet aggregates which have been formed under the influence of ADP in citrated plasma.

If the platelets made free by RA 233 from the aggregates are centrifuged and resuspended in platelet-free plasma, aggregation takes place again after addition of ADP

To find out some more details on the mechanism of the inhibitory effect of RA 233 we have incubated Na_2EDTA platelet-rich plasma at room temperature for 3 hours in the

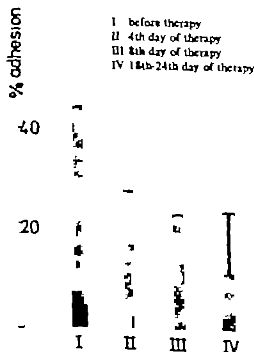
* Supported by the Deutsche Forschungsgemeinschaft

The pyrimidopyrimidine was made available by courtesy of Dr Karl Thomae GmbH, Biberach/Riss, Germany

Table I

Adhesion of platelets in citrated blood after intravenous administration of 2,6-Bis(diethanol-amino)-4-piperidino-pyrimido-(5 4-d)pyrimidine The values give the percent adherent platelets as measured by the Hellem-method.

test person	control	10'	30'	60'	120'	240'
		after application of RA 233				
100 mg RA 233						
H.R.	32 %	10 %	0 %		6 %	19 %
V.S.	28 %	5 %	7 %	4 %	0 %	0 %
R.S.	[illegible]6 %	14 %	9 %	22 %	4 %	–
150 mg RA 233 i.						
B.T	34 %	4 %	16 %	4 %		2[illegible] %
H.R.	24 %		12 %			16 %
D.W.	19 %	0 %	14 %	8 %	[illegible]8 %	6 %



Laboratory Controls

	I	II	III
creatinin	0.8	0.55	0.9 mval
ur	29	29	31 mg %
bilirubin	0.65	0.45	0.54 mg %
SGOT	8.2	17 4	17 4 IU
SGPT	5 8	16.6	13.2 IU
alkal. phosph.	136	188	157 IU

Figure 1

Platelet adhesion during therapy with RA 233 Adhesion was measured by the Hellem-method before therapy on the 4th, 8th, and between the 18th and the 24th day of therapy The laboratory controls of creatinine urea, bilirubin, SGOT SGPT and alkaline phosphatase were performed before therapy and on the 4th and 8th day of therapy

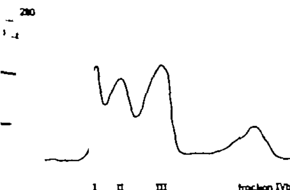


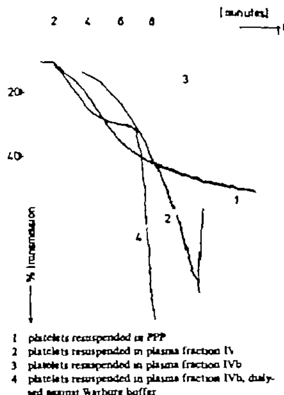
Figure 2

Chromatography of platelet-poor EDTA-plasma on Sephadex G-200 after incubation with 10^{-4} M RA 233.



Figure 3

Chromatography of platelet-poor EDTA-plasma on Sephadex G-200.



1 platelets resuspended in PPP
2 platelets resuspended in plasma fraction IV
3 platelets resuspended in plasma fraction IVb
4 platelets resuspended in plasma fraction IVb, dialysed against Warburg buffer

Figure 4

The inhibition of calcium-induced platelet aggregation by plasma fractions.

presence of 10^{-4} M RA 233 after centrifuging the plasma was applied to a column of Sephadex G-200 previously equilibrated with 5 10^{-3} Tris-buffer pH 7 4 The plasma proteins were eluted with the same buffer By this procedure 4 fractions of plasma proteins were obtained. As RA 233 is deep yellow the RA 233-containing fraction could easily be detected and isolated From analogous investigations of Schumacher (unpublished data) we may assume that the fraction IVb which contains RA 233 consists of a polypeptide having a molecular weight of about 16,000 to 25,000 (*Figure 2*).

Further investigations on the amino acid contents of this fraction are still in progress. Control plasmas which had been subjected to gel filtration on sephadex G-200 without RA 233 resulted in a similar elution curve with 4 fractions (see fraction IVa in Figure 3) It may be of interest that after incubation of plasma with acetylsalicylic acid and subsequent chromatography this drug is found in fraction IV too The effect of the different eluted protein *fractions on platelet aggregation* was subsequently investigated. Calcium was chosen as aggregating agent since the fractions were derived from gel filtration of Na_2 EDTA plasma. The mechanism of calcium-induced platelet aggregation has been described in *Klinische Wochenschrift 45* 931 1967 If normal platelet rich plasma is centrifuged and the platelet sediment resuspended in normal platelet-poor *plasma aggregation occurs after calcium is* added (Figure 4 n 1) If the platelet-free plasma is replaced by fraction IVa, strong aggregation by calcium ions takes place though the protein content of the fraction is only 25 %

of the protein content of platelet-poor plasma (Figure 4 curve n.2) Fraction IVb which is obtained after gel filtration of plasma in the presence of RA 233 brings about total inhibition of the calcium-induced aggregation as can be seen from Figure 4 curve n.3 Curve n 4 (Figure 4) shows the effect of fraction IVb after dialysis against Warburg buffer pH 7 4 The somewhat weaker aggregation as compared with the aggregation obtained with platelets resuspended in fraction IVa may depend on the fact that some of the polypeptide has permeated the membrane during dialysis. The inhibitor separated from the polypeptide by dialysis seems to be unchanged. After concentrating, it is able to inhibit platelet aggregation again. These findings indicate that the whole molecule of RA 233 may be responsible for its aggregation inhibiting action. Like other inhibiting substances, RA 233 possesses an aromatic system by which it is able to bind to plasma factors by means of hydrogen bridges.

Acetylsalicylic acid has a inhibiting effect on the oxidative metabolism of the platelets besides its effect on plasma factors. If platelets are incubated in the presence of acetylsalicylic acid the ATP-content of the platelets decreases. From recent investigations of Schneider (*Habilitationsschrift* Köln 1970) we know that the oxidative metabolism of the platelets may be responsible for the maintenance of their functional integrity while the glycolytic pathway might supply the oxidative pathway with substrates. Inhibition of the oxidative metabolism e.g. by malonate amytal, antimycin and oligomycin results in a rapid decrease of the ability of the platelets to aggregate

Our investigations have shown that RA 233 does not significantly modify the metabolism of the platelets. Platelet-rich plasma was incubated for 3 hours at 4 C and 37°C in the presence of 10^{-4}M RA 233 and the substrates ATP ADP lactate and pyruvate were determined in the platelet sediments (methods in Bergmeyer H U *Methoden der Enzymatischen Analyse* Verlag Chemie Weinheim, 1967) The values obtained (Table II) indicate that there are no significant differences between the samples which had been incubated with RA 233 and the controls. The somewhat lower concentrations of lactate in the RA 233-samples might depend on an activated oxidative metabolism by which an increased consumption of lactate would occur In the experiments done at 37°C the higher content of ATP in the RA 233-sample as compared to the control would be in agreement with this assumption

Furthermore our experiments demonstrate that incubation of platelets at 4 C has an effect on the oxidative metabolism by which the ATP-content of the platelets decreases This is in agreement with the findings of Schneider

Table II

The effect of RA 233 on the metabolism of platelets Incubation of platelet-rich plasma was performed both t 4°C and 37°C in the presence of 10^{-4} RA 233 for 3 hours The mean values obtained from 5 normal persons are given.

	ATP	ADP	Lactate	Pyruvate	ATP/ADP
	(concentration of substrates in µm/10 ¹ platelets)				
analysis directly afte preparation	7 9 ± 1 6	3 6 ± 0.7	14 0 ± 4 1	1 1 ± 0.3	2.03
control 4 C	6.2 ± 1 6	3 6 ± 0 7	19 1 ± 4 2	0.8 ± 0.	1 74
RA 233 4 C	6.3 ± 1 3	3.5 ± 0 3	15 4 ± 7 6	1 0 ± 0 2	1 80
control 37 C	7 3 ± 1 0	3.3 ± 0.5	19 9 ± 5 5	1 4 ± 0 2	2.19
RA 233 37°C	7 9 ± 1 2	3 6 ± 0.5	16.6 ± 6 9	1 3 ± 0.2	2.22

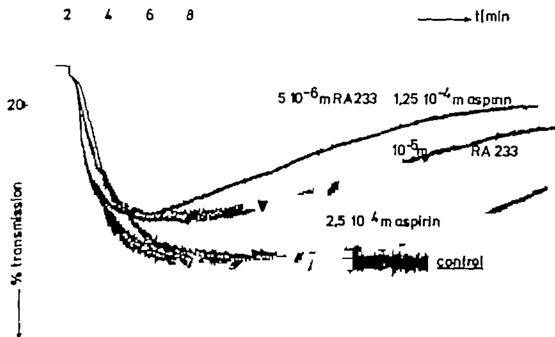


Figure 5

Aggregation inhibiting effect of acetylsalicylic acid + RA 233 Aggregation was induced in EDTA-plasma by calcium-ions.

(*Habilitationsschrift* Koln 1970) who found that the so-called energy sparing preparation of platelets at 4 C brings about an inactivation of the cold-sensitive oxidative phosphorylation. By this cold-induced inactivation via a Pasteur effect the glycolytic flow rate is increased about 203 fold. This increase nevertheless cannot keep the ATP content of the platelets constant

Because of the findings by which acetylsalicylic acid as well as RA 233 affect aggregation probably by interacting with the same fraction of plasma proteins, it was of special interest to examine if the simultaneous administration of acetylsalicylic acid and RA 233 might result in a potentiation of the aggregation inhibiting effects In Figure 5 the results of preliminary in vitro investigations are reported. As the aggregation curves clearly indicate there is a potentiation of the effects of acetylsalicylic acid and the pyrimidopyrimidine RA 233

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DISCUSSION

H.W SCHRÖTER In addition I should like to mention that the above named substances are not only more effective than dipyridamole as inhibitors of platelet function but also have very little effect on the circulation For example equal doses applied intravenously will change the blood pressure in dogs as follows

Dipyridamole reduces by maximally 52 mm Hg for about 1 hour

RA 233 reduces by maximally 23 mm Hg for about 14 minutes

VK 744 reduces by maximally 6 mm Hg for about 1 minute

So I think we may be in a position to use much higher doses of these drugs in human beings.

K. BREDDIN I want to show a slide (Figure 1) which corresponds with one shown by Dr Reuter here we have used practically the same procedure but we have incubated plasma with C labeled RA 433 after five minutes incubation the plasma was separated on a Sephadex G-200 column. The radioactive material is partly found in the albumin fraction and the largest amount is found in the fourth fraction as Dr Reuter has just shown with a very similar technique

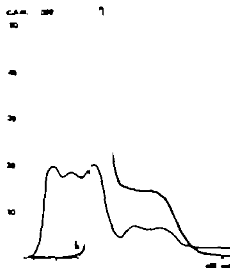


Figure 1

Platelet-poor plasma as incubated for 5 minutes with C-labelled RA 433 and afterwards separated by gel chromatography Curve represents the elution pattern of the proteins, curve b show the corresponding CPM of the [1] C label

C. OTHER SUBSTANCES

40 EFFECT OF DEXTRANS ON PLATELET FUNCTION

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Dextran has in numerous reports been shown to have an antithrombotic effect. It has in clinical practice mainly been used to reduce the frequency of *postoperative thromboembolic complications* (for references see Bygdeman, 7) The mechanism for the antithrombotic effect of dextran is complex. In this presentation I will restrict myself to the possible effect of dextran on platelet function.

Dextran infusion to normal individuals is followed by a marked decrease in ADP induced platelet adhesiveness as illustrated in Figure 1 This effect of dextran is molecular weight dependent since dextran 70 (dextran preparation with a mean molecular weight of 70,000) is more effective per gram dextran infused than dextran 40 (mean molecular weight 40,000) in reducing ADP-induced platelet adhesiveness (‿,8,9 11 12,16). The maximal effect is observed 2 to 5 hours after the end of infusion and is not related to plasma dilution. This has been further evaluated by Weiss (16) who demonstrated that a similar dilution induced by an infusion of albumine did not influence platelet adhesiveness. ADP induced platelet aggregation measured with a modification of the turbidometric method of Born (4) was decreased following dextran infusion (9). Arfors et al. (1) have also demonstrated that dextran inhibits platelet aggregation *in vivo* at the site of a biolaser induced endothelial trauma.

Dextran added to platelet-rich plasma *in vitro in concentrations likely to occur in vivo* ($\leq$ 2 %) does not, however decrease either ADP-induced platelet adhesiveness or aggregation (8,9 16). These results suggest that the effect of dextran infusions on platelet adhesiveness and *aggregation at least to some extent* may be due to a colloid-pharmacological effect on plasma factors *in vivo* responsible for normal platelet adhesiveness and aggregation

Addition of dextran to platelet-rich plasma *in vitro* does not influence collagen-induced platelet aggregation or the release of ADP (16). The release of 5-hydroxytryptamine from blood platelets induced with collagen or with thrombin is not either affected by dextran (Figure 2) (6). The mechanical strength of the platelets, however appears to be increased following addition of dextran as shown in Figure 3 (5 6).

Addition of dextran to human platelet-rich plasma can induce a slight decrease in optical density which cannot be explained by dilution (9 13 14). The same decrease in optical density can, however be induced with human albumin and gamma-globulin (9) and this effect may be secondary to phase boundary phenomena such as adsorption or refraction.

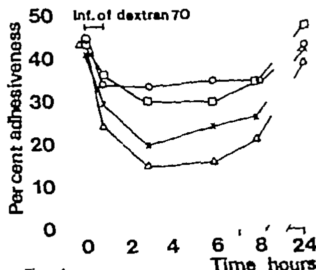


Figure 1

Effect of infusions of dextran 70 in doses from 100 to 1,000 ml on ADP-induced platelet adhesiveness. Amount of dextran infused.
○ 100 ml, □ 250 ml, 500 ml, △ 1000 ml.

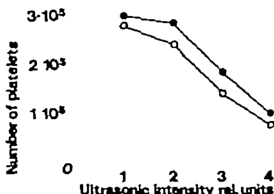


Figure 3

Effect of dextran 153 on the number of platelets surviving an ultrasonic stress.

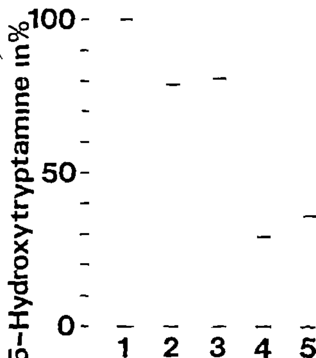


Figure 2

Platelet concentration of 5-hydroxytryptamine in % of control (1) and following addition of 0.1 ml/ml plasma of connective tissue suspension diluted 1/5 (2 and 3) and 1/2 (4 and 5). In experiment 2 and 4 dextran 70 was added in final concentration of 10 mg per ml plasma.

In studies on rabbit platelets Westerholm (17) has shown that small concentrations of dextran (10^{-8} – 10^{-4} g/ml) could induce a release of 5-hydroxytryptamine (5-HT) from the platelets, while higher concentrations were inhibitory. This release was temperature and calcium dependent and it was suggested that enzymatic mechanisms were involved. Westerholm also reported that the release reaction was inhibited by ninhydrin a NH_2-group inhibitor and by allicin a SH-group inhibitor which is compatible with such a suggestion, since it is known that many enzymes depend on amino and sulfydryl groups for their activity. We have not been able however (Bygdeman and Johnsen, unpublished observations) to observe any 5-HT releasing effect of these small concentrations of dextran in our own studies on human platelets.

Paterson and Dhall (15) have recently presented results which indicate that dextran may release small amounts of ADP from human platelets and suggested that this may be one explanation for the antithrombotic effect. In order to find out if dextran can induce a platelet release reaction as defined by Grette (14bis) we have chosen also to study the effect of higher concentrations of dextran on platelet content of 5-HT. These results have been summarized in Tables I and II. In the first series of experiment human citrated platelet-rich plasma (PRP) was incubated with tracer amounts of

[14] C 5 HT for 1 hour at 37°C. After the end of the incubation period more than 95 % of the radioactivity was taken up by the platelets. Dextran 70 was then added in 3 concentrations from 1 to 2 % and after mixing, the plasma was reincubated at 37°C for 0 to 30 min.

At the end of the incubation period EDTA was added in a final concentration of 0.077 M and the PRP cooled and centrifuged at 700 g at 4 C for 15 minutes. The platelet pellet was washed twice and platelet radioactivity measured as previously described (10). The results indicated (Table I) that incubation with dextran caused a slight decrease in measured platelet radioactivity. This decrease could be fully explained by the finding of an increased number of platelets remaining in the supernatant platelet-poor plasma after the first centrifugation and no decrease in radioactivity could be found if the results were correlated to the number of platelets remaining after centrifugation and washing. These results could be fully confirmed in the second series of experiments, which included measurements of the total amount of platelet 5-HT utilizing the method of Bertler (3) and which are summarized in Table II. At the present state it must therefore be concluded that no convincing evidences are available in favour of the suggestion that dextran in clinical doses can induce a platelet release reaction.

Table I

Effect of dextran on platelet content of C 5-hydr xytryptamine. All values are given in per cent of the control value at the start of incubation. Incubation temperature 37 C. Mean values ± SD (= 3)

Incubation time min	Dextran 0	1	Concentr tion % 1.5	2
0	100 ± 0	108 ± 6	101 ± 3	94 ± 7
5	104 ± 2	100 ± 4	94 ± 5	92 ± 3
10	101 ± 3	102 ± 1	93 ± 5	91 ± 5
30	101 ± 3	94 ± 11	95 ± 11	92 ± 10

Table II

Effect of dextran 70 on platelet content of 5-hydroxytryptamine. Plasma volume 5 ml. Concentration of dextran 2 %. Incubation time 30 min. Temper ture 37 C. Mean values of 11 experiments

	Total platelet count		5 HT total amount µg		5 HT µg/10 platelets	
	Control	Dextran	Control	Dextran	Control	Dextran
Mean	1.05 10⁹	0.89 10⁹	0.59	0.55	0.57	0.63
± SD	± 0.19	± 0.12	± 0.11	± 0.05	± 0.18	± 0.14

Acknowledgements

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41 PLATELETS AND CONTACT ACTIVATION OF BLOOD CLOTTING INFLUENCE OF SOME AGGREGATION INHIBITORS

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We would like to present a few remarks on some drugs acting on platelet aggregation as related to the possible role of platelets in the contact activation of plasma While working on surface activation problems, we observed that an enhancement of the formation of activation product could be obtained in hyperlipaemic plasma provided that the glass activation of the latter occurred in presence of platelets (?) This finding was explained assuming that platelets, in addition to their well known properties, could also play a role in the initiation of clotting, namely in the contact activation phase In fact, it was suggested that platelets are involved in the surface activation and in the formation of activation product as being probably responsible fo the neutralization of a normally existing inhibitor of factor XII activation (2,5).

The existence of this inhibitor in Hageman plasma had already been postulated by previous workers but later on it could be demonstrated also in normal plasma (5) No data are available which could explain what functional state of the platelets is required for the appearance of this neutralizing ability

In our previous experiments ( ) hyperlipaemic conditions parallely stimulated platelet adhesiveness to glass and platelet aggregation by ADP Therefore we tried to investigate to what extent the activity of platelets neutralizing the Hageman inhibitor was related to the developing of stickiness and aggregation phenomena.

Our studies, based on this tentative approach, are still in progress, but we will report here some preliminary results obtained with different drugs acting as inhibitors of platelet aggregation.

In our original working hypothesis, if platelet participation to the activation of surface factors was in some way related to their adhesive and aggregating tendency we could expect that a number of anti aggregating agents, might also be able to hamper the formation of activation product n platelet rich plasma (3) It is clear that this kind of approach to anti-aggregating agents could also have some clinical implications. The first agent tested on patients was poly-unsaturated phosphatidylcholine we found that this phospholipid, containing a high percentage of linoleic acid, was an excellent inhibitor of platelet adhesiveness to glass and was also able to reduce significantly platelet aggregation measured both with Breddin s method (1) and photometri-

Table I

Polyunsaturated Phosphatidylcholine
500 mg daily i. 15 days, 25 patients

Platelet Stickiness %		Platelet Aggregation % (ADP)		Platelet Aggregation (Breddin)
before	after	before	after	
36.27	24.68	40	32	reduced significantly in
± 3.45	± 4.67	± 3.69	± 3.47	24 cases
$p < 0.01$		$p < 0.01$		

Blood Coagulation	Fibrinolysis
No antithrombin effect Anti contact-activation effect	Enhanced only through effect on lipids.

Table II

Duodenal Polysaccharide
200 mg daily i.m., 18 days, 20 patients

Platelet Stickiness %		Platelet Aggregation (ADP		Platelet Aggregation (Breddin)
before	after	before	after	
		39 %	28 %	reduced in 16 ca-
37.40±3.28	28.45±4.27	± 4.24	± 3.15	ses on 20
$p < 0.01$		$p < 0.01$		

Blood Coagulation	Eugl. Fibrinolysis (lysis area)	
Slight antithrombin effect	86	148
Antithromboplastic effect	± 5.4	± 10
Anti-contact activ. effect	$p < 0.01$	

cally after ADP addition (6) to a constant number of platelets. Phosphatidilcholine at single (3 4) or repeated administration (Table I) worked also as an inhibitor of the activation of surface factors, provided the contact reaction occurred in platelet-rich plasma. The presence of platelets is essential for the anti-activation activity of this phospholipid to be disclosed.

Two heparinoid substances were tested thereafter: a sulphonic polysaccharide from the duodenal wall and a semisynthetic polysulphonic ester of low molecular weight, both effective hypolipidaemic and fibrinolytic agents. These agents showed also a definite anti-adhesive and anti-aggregating activity on platelets. It is obvious that heparinoids possess a much broader activity on blood coagulation than most agent tested. Nevertheless, using a three stage thromboplastin activation method (5) we could state that they act not only as very

T ble III

Sodi m Betabenzalbutyrate 400 mg daily i.m., 18 day 18 patients				
Platelet Stickiness %		Platelet Aggregation % (ADP)		Plat let Aggregation (Breddi)
before	after	before	after	
34.03	21 90	44	32,5	reduced 13 ca
± 3.38	± 4 87	± 3.18	± 4 2.	ses on 18
$p < 0{,}01$		$p < 0{,}01$		

Blood Coagulation	Luglobulin F brinolysis (lysis area)	
	before	after
No evident effect	86	90
On contact activation	± 5	± 6.5
	$p > 0{,}05$	

moderate antithrombin and anti-thromboplastin inhibitors, but also as inhibitors of contact activation in platelet-rich plasma (Table II).

Other agents were tested thereafter and namely dipyridamol, clofibrate and sodium beta-benzal butyrate. All of them are strong inhibitors of platelet adhesiveness, especially the latter (Table III) but none showed a significant activity in the contact activation of platelet-rich plasma

Our experiments with aggregation inhibitors did not confirm entirely our initial assumption, since some strong anti-aggregating or anti-adhesive substances had no effect in the contact activation of platelet-rich plasma. Among the agents tested, the poly-unsaturated phospholipid and the low molecular weight heparinoids *can couple anti-aggregating properties* with the ability of inhib ting the contact activation of platelet-rich plasma

These findings suggest that the ability of platelets to cooperate to contact activation does not necessarily develop parallely to their becoming adhesive and aggregated. In fact inh bition of stickiness and aggregation does *not always result in an impairment of their* part cipation to the contact reaction.

The agents able of coupling anti-aggregating and anti-contact effect show some significant physico-chemical features. Phosphatidilcholine is a molecule with strong polar properties and with marked effect on membrane phenomena heparinoids carry strong negative charges, possibly contributing to reciprocal repulsion of platelets

The activity of platelets in the contact phase seems therefore to be prevented by substances with certain physico-chemical properties and being also able to make platelets less sensitive to aggregating stimuli. The same ability seems not necessarily possessed by other anti-aggregating gents, which do not act on the contact phase

Studies are in progress in our laboratory as to a better elucidation of the physiological *condition of platelets during their interferen e* with the contact reaction, and to the identification of new substances with anti-aggregating and anti-contact activation properties.

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42. SOME EFFECTS OF MUCOPOLYSACCHARIDE STAINS ON PLATELETS

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I want to report some curious observations concerning the effect of mucopolysaccharide stains on platelets in platelet-rich plasma. Almost at random I selected five stains for mucopolysaccharides. Lanthanum nitrate and ruthenium red are used in electron microscopy and brilliant cresyl blue toluidene blue and alcian blue are used in light microscopy Each of these stains added to platelet-rich plasma at a sufficiently high concentration completely inhibits platelet aggregation when ADP or thrombin or adrenaline or collagen are subsequently added. At lower concentrations of the salts of lanthanum and ruthenium inhibition can be shown to be competitive Thus exactly the same tracing can be obtained by lowering the amount of ADP added or by increasing the amount of lanthanum. This suggests that the inhibitory effect of these comounds is exerted on or through a normal physiological pathway

If these stains are diluted further none has any effect except alcian blue (AB). At high concentration (4 mg/ml) AB has no effect itself but inhibits aggregation if ADP or other aggregating substances are subequently added. AB (0.5 mg/ml) added to stirred platelet-rich plasma itself causes aggregation after a delay The tracing looks similar to that produced by collagen and indeed an aggregating agent is shown to be released by AB. At still lower concentration (0.05 mg/ml) AB itself again has no effect but added before ADP it causes a marked potentiation of the ADP response

There is no firm evidence that these five stains used by histologists stain only mucopolysaccharides (MPS) and particularly at physiological pH indeed their ability to stain is known to be non-specific. Nevertheless, since five stains with very different chemical structures are all known to stain MPS and all have an effect it is possible that they have this effect by virtue of staining and thereby altering platelet mucopolysaccharides. It then would follow that MPS in the platelet when altered by "staining" itself influences platelet aggregation.

The ability of AB itself to produce release seems to be a quite different process from the inhibition of aggregation by other compounds and requires further investigation. As pure speculation I wonder if AB has an effect on the membrane of the platelet granule

All these five staining compounds carry a positive charge and are able to neutralise heparin which carries a negative charge AB added to washed human red cells in saline causes them to aggregate This observation might suggest that at least with red cells when AB is added, the red cells are stained and this produces a simple charge effect leading to mutual attraction between the cells. However the degree of aggregation is greatly inhibited by adding plasma or by decreasing the temperature This suggests that there is a relatively complicated interaction between AB and red cells. This is under investigation. Some preliminary results are reported in *J clin. Path. 23* 784 1970.

43 EFFECT ON PLATELET BEHAVIOUR OF BETA BENZAL BUTYRIC ACID

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The preliminary results obtained with a new hypolipaemic compound (β-benzal-butyric acid (BBA) are reported here with emphasis on its influence on platelet behaviour both *in vitro* and *in vivo*

BBA is a phenyl derivative of 5-carbon branched chain acids (MW 198) which has been shown to inhibit *in vitro* the synthesis of both cholesterol and fatty acids from acetate whereas the synthesis from mevalonate is hardly affected (1 4,5) It is supplied as sodium salt which can be either given orally or injected intravenously as acid

Influence of BBA on Platelet Aggregation in vitro

Platelet aggregation was studied at 37°C by a turbidometric method, using an EEL Platelet Aggregation Meter 0.4 ml of citrated platelet-rich plasma (PRP) ($3\text{-}5 \times 10^8$ platelets/ml) were incubated for one minute with either buffered saline (control) or various concentration of a buffered solution of BBA. Various concentrations of the aggregating agents (ADP Sigma adrenaline Sigma and a collagen suspension-Sigma) were added after one minute recording the change in transmission automatically on a Servomatic (RE 511) Goerz Recorder

The general plan of the experiments with each aggregating agent was to test increasing concentrations of BBA, of which we are evaluating a possible inhibitory activity with two standard concentrations of the aggregating agents.

ADP

PRP of 8 normal subjects was tested with two standard concentrations of ADP (2 and 0 5 μg/ml) (Table I) With the higher concentration of ADP (2 μg/ml) five control samples showed two waves of aggregation in 3 other samples, the second wave was not discernible in the tracing, but aggregation was irreversible. Inhibition of aggregation was evident with concentrations of BBA ranging from 200 to 700 μg/ml as judged by a decrease in transmittance in comparison with the control, by the presence of disaggregation and by the absence of the second wave of aggregation.

With the lower concentration of ADP (0.5 μg/ml) 2 waves of aggregation were evident in all the control samples.

BBA, in concentrations ranging from 120 to 700 μg/ml, caused in all the cases a decrease in light transmittance accompanied by disaggregation and absence of the second wave.

The degree of inhibition was slightly higher

Table I

Effect of beta-benzal-butyric acid (BBA) on ADP collagen and adrenaline induced platelet aggregation. The figures indicate aggregation response expressed as per cent aggregation" relative to the control (mean values)

BBA μg/ml	ADP μg/ml n = 8			Collagen mg/ml n = 7		Adrenaline μg/ml n = 6		
	2	0.5	Second wave	50	15	20	5	Second wave
650	51	43	–	20	14	22	20	–
500	63	50	–	30	17	27	25	–
300	74	66	–	47	25	28	24	–
200	78	70	–	55	30	40	32	–
100	83	80	±	64	52	41	50	±

n = number of experiments.

than that produced by 2 μg/ml of ADP at corresponding concentration of BBA, as shown in Table I

Collagen

A standard collagen suspension (50 mg/ml) caused irreversible platelet aggregation in 7 control PRP after a delay of 2 3 min

In the presence of different concentrations of BBA, there was a marked inhibitory effect which was progressively lost as the concentration of BBA has decreased. Both the rate and the maximal degree of platelet aggregation seemed to be affected as judged by the observation of the aggregation curves. The intensity of the inhibition was more marked and evident with lower concentrations using the weaker collagen suspension (15 mg/ml) (Table I).

Adrenaline

Adrenaline at standard concentrations of 20 and of 5 μg/ml caused 2 waves of platelet aggregation in 6 control PRP

The addition of BBA to PRP was followed by a marked decrease of light transmittance and disaggregation in 5 samples, with complete inhibition of the second wave of aggregation The inhibition was less effective with decreasing concentrations of BBA and was practically lost at a concentration of 50 μg/ml (Table I)

Platelet factor 3 (PF3) availability

These experiments were carried out to see whether BBA added to PRP affected the availability of PF3 The test for PF3 availability used in the present study was that described by Hardisty and Hutton (2) using kaolin as activator

Various concentrations of BBA were added to PRP and incubated with kaolin (20 mg/ml) for 20 minutes with gentle agitation.

The clotting time obtained after addition of $CaCl_2$ was compared with the control in a

Table II

Effect f beta-benzal-butyric acid (BBA) on platelet factor 3 availability induced by kaolin.

BBA Concentration μg/ml	Mean Clotting time, sec. (n = 6)	S.D.
650	52.6	3.9
300	45.4	2.6
200	42.1	3.4
	39.2	2.6

0.2 ml of PRP were incubated for 20 min tes at 37 C with kaolin (20 mg/ml) and BBA or buffe with gentle agitation The clotting time was recorded after addition of M/20 $CaCl_2$.

similar system where buffer replaced BBA.

Concentrations of BBA ranging from 700 to 100 μg produced a definite prolongation of the clotting time compared with the control plasma (Table II).

In vivo experiments

The evidence of inhibition of platelet aggregation obtained in our *in vitro* studies suggested that BBA might be tested to see whether this effect was evident *in vivo* at doses employable in man

It was therefore decided to try and find the effective dose of BBA, the rate of onset of its effect and whether or not the effect was sustained. Accordingly 18 normal subjects not taking aspirin or related drugs were studied with respect to collagen and ADP-induced platelet aggregation before and after a single dose of BBA.

The experiments of platelet aggregation were carried out as described before particular attention was paid to carry out the experiments at constant intervals after the collection of the blood (45 minutes) and to complete them within 90 min. Two standard concentrations of ADP (2 and 0.5 μg/ml) and of collagen (50 and 15 mg/ml) were used. The samples of PRP taken from the same subjects at intervals after the administration of BBA were rejected if the platelet count was not in the range of $\pm 0.5 \times 10^8$ platelets/ml of the original count, which was in any case in the range of $3-5 \times 10^8$ platelet/ml.

Intravenous administration

4 subjects were treated with 600 mg of BBA injected intravenously (single dose). A slight inhibition effect was evident only with the weaker concentration of ADP and collagen, and was completely lost after 90 min.

5 subjects were treated with 1,2 gr of BBA intravenously (single dose). While the effect on the aggregation induced by the strongest concentrations of ADP and collagen was questionable a definite inhibitory effect was evident and lasted for at least 4 hours with the weakest concentrations of ADP and collagen tried. With collagen the maximal deflection at 4 min and the rate of aggregation were decreased if compared with the basal values with ADP the second wave was absent and disaggregation was evident.

4 subjects were treated with 1,8 gr of BBA (single dose). Aggregation with the weaker concentrations of ADP and collagen was markedly impaired, and the inhibition lasted for 5-6 hours. The pattern of the aggregation curve was similar to that observed with 1,2 gr of BBA.

Oral administration

From the results obtained with the intravenous administration and from absorption studies in animals, it was assumed that an oral dose of 2 gr of BBA should be suitable to produce a consistent inhibition of platelet aggregation with a single dose

5 subjects were treated with a single dose of 2 gr BBA 4 hours after their last meal and studied thereafter every 2 hours for 8 hrs. Consistent inhibition of platelet aggregation (70 % or less of basal aggregation) was obtained in only 2 cases with the concentrated collagen suspension in 4 cases with the diluted collagen suspension in no case with μg/ of ADP and in 5 cases with 0.5 μg of ADP The inhibition, when present, was evident up to 8 hours, but was completely lost after 24 hours.

The administration of BBA was in any case very well tolerated and no clinical or laboratory evidence of side effects was noted in this small series of patients treated with a single dose

Conclusions

Evidence has been obtained of an inhibitory action of BBA on platelet aggregation and PF3 availability when added to PRP in the test tube Moreover from our preliminary studies, it seems that BBA is capable of inhibiting collagen- and ADP induced platelet aggregation after administration in humans. The effect is short-lasting and its decline seems to parallel the elimination of the drug from the circulation. The dose used in our preliminary experiments to inhibit platelet aggregation is undoubtedly large, but it is free of side-effects. On the other hand it is possible that administration of spaced doses may allow a reduction of t total daily dosage.

The mechanism by which BBA influences platelet behaviour is entirely speculative at the moment. The inhibition of collagen-induced platelet aggregation, of the second wave of both ADP and adrenaline induced aggregation, and of PF3 availability induced by kaolin suggest that the effect of the drug may be on the platelet release reaction.

Another possible mechanism for the action of BBA on platelet behaviour could be related to platelet lipid metabolism. In contrast to erythrocytes and leukocytes, platelets contain the enzymes for fatty-acid synthesis (3). Since BBA has been shown to inhibit acetyl-CoA ligase (4) which is involved in the early phases of fatty-acid syntehsis from acetate, its inhibitory action on platelets may be linked to alterations in the structure and composition of platelet membrane. However this hypothesis is hardly compatible with inhibition developing rapidly (within one minute incubation). The possibility that the alteration of platelet behaviour induced by BBA may be related to an effect of the drug on some plasma component rather than on the platelets cannot be ruled out at the present stage of investigations.

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44 EFFECT OF NIMERGOLINE (F.I. 6714) ON HUMAN PLATELET AGGREGATION IN VITRO*

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Nimergoline (F.I. 6714) (Figure 1) is a new vasoactive compound recently introduced for the clinical trials. A powerful adrenolytic effect was demonstrated in preclinical studies.

Inhibition by nimergoline of ADP and adrenaline-induced human platelet aggregation was studied *in vitro*. Platelet aggregation at 37°C with continuous stirring was measured by a turbidimetric method using the platelet aggregation meter (EEL 169) and the changes in optical transmittance were graphically recorded. Inhibition of ADP (1.2 μM) induced platelet aggregation was evident at nimergoline concentration of 3 μM and increased until a maximum value of 80% with a concentration of 320 μM (Figure).

Adrenaline (80 μM) induced platelet aggregation was completely inhibited by equimolecular doses of nimergoline (Figure 3): the same was observed for the potentiating effect of adrenaline on ADP-induced aggregation. These results show that at equivalent molecular concentrations, the nimergoline inhibiting effect is more powerful on adrenaline than on ADP induced aggregation. Nimergoline was able to prevent the appearance of the normal second wave of aggregation induced either by ADP or by adrenaline (Figure 4): the rapidity of disaggregation was proportioned to the concentration.

Moreover a marked disaggregation was also observed after addition of nimergoline when the aggregation appeared irreversible (Figures 5 and 6).

In vivo studies in animals and in man are in progress to define the therapeutic value of this drug as an antiaggregating agent.

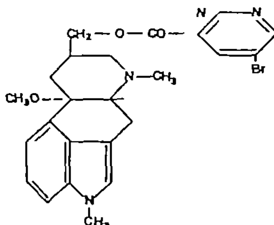


Figure 1
Nimergoline: Structural Formula.

*Nimergoline was supplied by Farmitalia, Research Laboratories, Milano, Italy

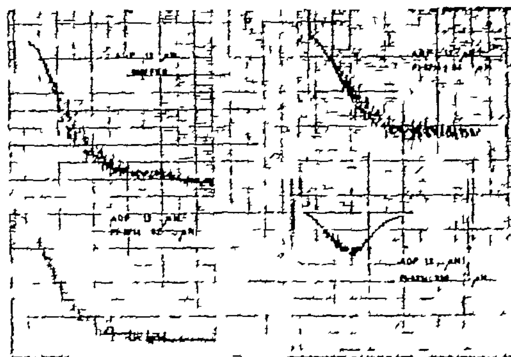


Figure 2

Inhibition of ADP-induced platelet aggregation, by increasing concentrations f nimergoline.

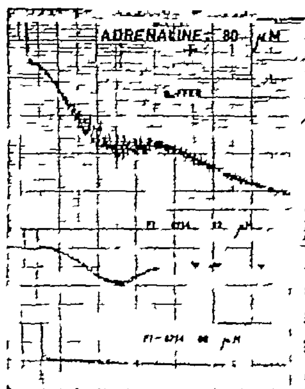


Figure 3

Inhibition of Adrenaline-induced platelet aggregation by increasing concentrations of nimergoline.

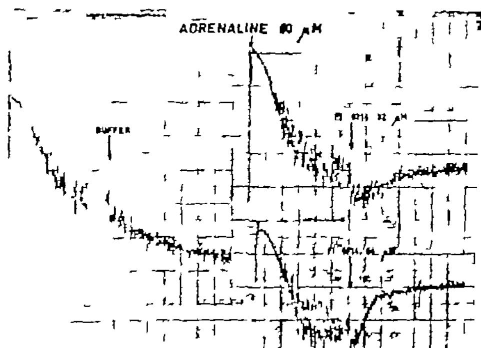


Figure 4

Prevention of Adrenaline-induced second wave of aggregation by addition of different concentrations of nicergoline

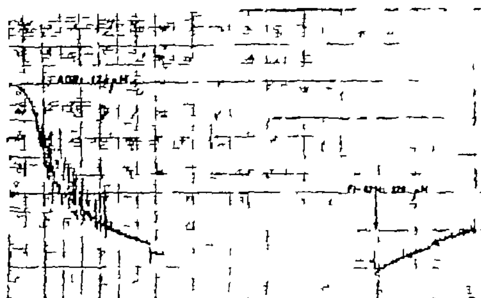


Figure 5

Disaggregation by addition of nicergoline when ADP-induced aggregation appeared irreversible for more than 10 minutes.

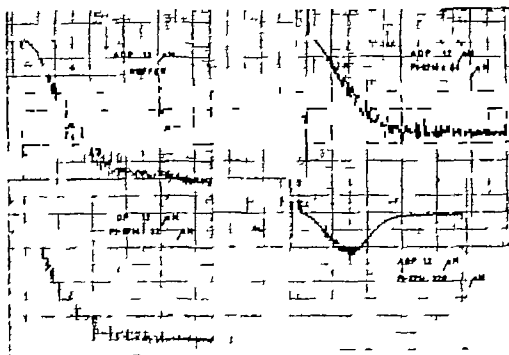


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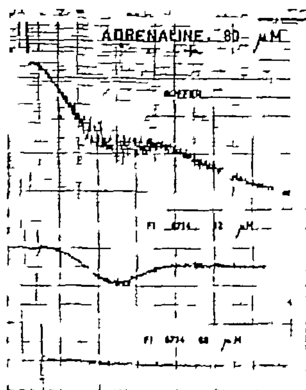


Figure 3

Inhibition of Adrenaline-induced platelet aggregation by increasing concentrations of nimergoline.

Question N 14

IN WHICH CLINICAL CONDITIONS WOULD PHARMACOLOGIC INHIBITION OF PLATELET AGGREGATION BE USEFUL ?

45 H. GASTPAR The Inhibition of the Cancer Cell Stickiness by Pyrimido-pyrimidine Derivates Induced by Inhibition of Platelet Aggregation

46. S COCCHERI and F FIORENTINI Platelet Adhesiveness and Aggregation in Hypertensive Patients.

DISCUSSION

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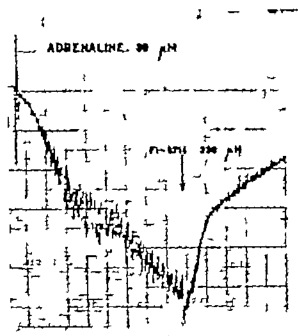


Figure 6

Marked disaggregation by addition of nicergoline after second wave of adrenaline-induced platelet aggregation

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45 THE INHIBITION OF THE CANCER CELL STICKINESS BY PYRIMIDO-PYRIMIDINE DERIVATES INDUCED BY INHIBITION OF PLATELET AGGREGATION

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The first step in the formation of metastasis is the interaction between blood-borne cancer cells and the vascular endothelium. Circulating cancer cells display a more or less prominent tendency to stick on the intact endothelium of capillaries and venules. Once they have been lodged behind the arresting cell results a turbulence of the resisting blood flow (8). Thrombocytes and leucocytes are attracted into this turbulence and preferably aggregate there Within few minutes they become enmeshed in a fibrin clot The sequence of these events may be visualized by intravital microscopy and microcinematography demonstrated at first by Wood et al. (14) and Gastpar et al. (4).

The lodgement of cancer cells on the endothelium depends mainly on their "stickiness (3) a specific indigenous physico-chemical property of the cell surface (13), that is related to its thromboplastic activity (2,10,11 15) Platelets also may aggregate on sticky cancer cells during their circulation (5). Such a mass attaches more readily on the endothelium.

These experimental results briefly discussed suggest that the development of metastases from blood-borne cancer cells is closely connected with intravascular clotting (13). Since the impressive studies by Lawrence et al. (11 1) and Cliffton et al. (1) there is evidence that anticoagulant and fibrinolytic drugs interfere with the initial adherence of cancer cells to vascular endothelium and their enmeshment in a fibrin clot and may reduce the incidence of metastasis production of some experimental tumors (6 7)

Since thrombocytes play a very prominent role in the mechanism of cancer cell lodgement and subsequent thrombus formation we assumed that substances which block aggregation of platelets also help hinder lodgement of cancer cells. Dipyridamole (Persantin) and some other pyrimido-pyrimidine compounds have been shown to influence the platelet behaviour in vitro and in vivo (8 9). Therefore we tried by intravital microscopy in mesentery of rats whether some new pyrimido-pyrimidine compounds influence the sticking behaviour of circulating cancer cells (8 9) The mesentery of wistar rats of a body weight of 150- 00 g was dissected after induction with chloralhydrate Then ml of a suspension of 1×10^{4} fluorochromated Walker 256-carcinosarcoma cells were slowly injected into a polyethylene-catheter inserted into the jugular vein

Within 5-10 minutes after the infusion lethal

Table I

Effect of 5 pyrimido-pyrimidine derivatives on pulmonary tumor cell embolism in rats after intravenous transplantation of 1 10 Walker 256-carcinosarcoma cells in jugular vein

Substance	Dose	No. of died/treated rats	rat of died animals (%)
RA 8	3 mg/kg	19/40	48 %
RA 8	6 mg/kg	14/40	35 %
RA 8	10 mg/kg	8/30	27 %
RA 433	2 mg/kg	21/40	53 %
RA 433	4 mg/kg	15/40	38 %
RA 433	6 mg/kg	9/30	30 %
RA 433	10 mg/kg	3/15	20 %
RA 233	3 mg/kg	12/40	30 %
RA 233	6 mg/kg	3/30	10 %
RA 233	10 mg/kg	0/20	0 %
RA 255	3 mg/kg	10/40	25 %
RA 255	6 mg/kg	2/40	5 %
RA 255	10 mg/kg	0/40	0 %
VK 744	3 mg/kg	5/30	17 %
VK 744	6 mg/kg	1/30	3 %
VK 744	10 mg/kg	0/30	0 %
Controls		36/60	60 %

Dipyridamole Persantin

tumor cell embolism of the lungs resulted in 60 % of the control animals (Table I) This was caused by the blocking of pulmonary capillaries and arterioles by lodging and clumping tumor cells that may even produce occlusion of a complete pulmonary segment This lethal rate was diminished to 7 % by intravenous administration of 10 mg/kg Dipyridamole (RA 8) 5 minutes before the tumor cell infusion to 20 % by RA 433 and to zero by RA 33 RA 255 and VK 744 The effect of these test substances on lethal tumor cell embolism of the lung was quantified statistically by a probit analysis (8)

Criteria for the reaction of the test substances on the thrombocyte aggregation were the number of infused tumor cells that could be seen in a certain area (1 cm) showing a "fixed lodgement" to the vascular endothelium during a period of 30 minutes.

The number of the lodged tumor cells varied between 7 29 cells (mv=17) per animal in the controls (Table II) Dipyridamole and the other 4 pyrimido-pyrimidine compounds showed a dose response reduction of the fixed tumor cells in the same order of effectiveness as in the pulmonary embolism mortality Dipyridamole and RA 433 reduced the number of the lodged cells to a mean of 4 per animal Whereas a dose of 10 mg/kg of RA 233 and RA 255 and a dose of 6 mg/kg VK 744 stopped the fixed lodgement.

Dipyridamole and the 4 other pyrimido-pyrimidine derivates, especially the compounds RA 33 RA 255 and VK 744 are able to interfere with the dynamic interaction between sticky tumor cells and vascular endothelium, just as heparin but at an earlier stage pyrimido-pyrimidine compounds help to hinder the platelet aggregation on circulating sticky tumor cells and therefore significantly inhibit their lodgement on the endothelium by hindering the platelet aggregation around adherent cancer cells and blocking the subsequent thrombus formation. Further proof of this is that some of the tested substances prevented lethal pulmonary tumor cell embolism which occurred in 60 % of the controls

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T ble II

I ffect of 5 pyrimido-pyrimidine deriv tives on the l d lodgement of tumor cells t vascular endothelium in mesentery f t (are f observation 1 cm^2) after intravenous transplantation of 1 10^6 Walker 256-carcinosar oma cells ia jugular in

Substance	Dose	variation cells/animal	mean value cells/animal	No. of animals
RA 8	3 mg/kg	8-31	13	21
RA 8	6 mg/kg	3-14	17	6
RA 8	10 mg/kg	0-8	4	22
RA 433	2 mg/kg	5-21	14	19
RA 433	4 mg/kg	1 12	8	25
RA 433	6 mg/kg	0-8	5	21
RA 433	10 mg/kg	0-9	4	12
RA 233	3 mg/kg	0-14	8	28
RA 233	6 mg/kg	0-5		27
RA 233	10 mg/kg	0	0	30
RA 255	3 mg/kg	0-10	5	30
RA 255	6 mg/kg	0-4	1	38
RA 255	10 mg kg	0	0	40
VK 744	3 mg/kg	0-8	3	25
VK 744	6 mg/kg	0	0	29
VK 744	10 mg/kg	0	0	30
Controls		7-29	17	24

Dipyridamole Persantin

Thrombos Diathes haemorrh Suppl 28 119 1968

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46. PLATELET ADHESIVENESS AND AGGREGATION IN HYPERTENSIVE PATIENTS

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High haemodynamic pressure in the arterial tree is considered one of the main factors of atherogenesis. Continuous mechanical injury on the intima of arteries submitted to a high lateral tension is probably responsible for changes leading to fibrin deposition on the vascular wall.

In the context of a wider study on the so called "thrombogenic parameters" in hypertensive patients, we felt that the evaluation of platelet adhesiveness and aggregation could be one of the topics to be considered with special attention.

Our present report deals with a group of 43 patients of both sexes, all hypertensive, with diastolic values over 100 mm Hg. Patients with evidence of recent or remote thrombosis, or of heavy atherosclerotic changes, as shown by fundus oculi, sphygmography and haemodynamic response to amyl nitrite were excluded from this study.

Our preliminary results can be summarized as follows. Adhesiveness of platelets to glass after rotation of heparinized whole blood (3) and double platelet counting in a Coulter Counter was significantly higher than observed in a similar group of healthy controls (Table I).

The changes in platelet stickiness after saturated fat ingestion were also investigated in hypertensive and non-hypertensive patients (Table II). The increase of platelet adhesiveness was significant in both groups, but the correlation coefficient (r) between the increase in optical density and the increase in platelet stickiness was significant only in the non-hypertensive group, probably on account of the wider range of dispersion of adhesiveness values in the hypertensive patients.

After adrenaline infusion (Table III) the behaviour of platelet adhesiveness in hypertensive patients differs from that observed in normal patients. In fact, in hypertensive patients the lower doses of adrenaline failed to induce a significant augmentation of the platelet stickiness index, thus indicating a lower

Table I

Platelet adhesiveness to glass.

	N.	Adhesiveness index	Standard deviation
Controls	40	23,3	± 4,5
Hypertensive patients	43	27,25	± 8,9

Variance Analysis: $F = 6{,}98$, $p < 0{,}01$

Table II

Platelet adhesiveness to glass after f t ingestion.

	Increase adhesiveness-inde	P	Increase optical density		p
Controls (12)	9,52 ± 4 10	< 0,01	0,22 ± 0,16	0 75	< 0,01
Hypert nsive patient (10)	7 30 ± 8,06	< 0,05	0,29 ± 0,02	0 25	> 0,05

T ble III

Platelet adhesiveness (glass after adrenaline infusion

	Adrenaline γ/kg b.w. 0	5	10	20
Contr ls (20)	23 ± 4 2	29 ± 3,5	31 ± 4 7	31 ± 4,5
Hypertensive patients (20)	28 ± 4,8	29 ± 4 7	32 ± 5 9	36 ± 5,5

sensitivity of the platelets to exogenous adrenaline It is known that patients suffering from hypertension may be less sensitive to the hemodynamic effects of exogenous adrenaline This finding suggests that this kind of resistence can be observed also towards the ability of adrenaline of increasing platelet adhesiveness

The lower sensitivity of the platelets of hypertensive patients to adrenaline was confirmed in some in vitro experiments by means of a photometric method ( ) (Table IV) If adrenaline concentration in the test is increased from 40 to 80 micromole a marked increase of aggregation is observed with normal platelets, but no effect is obtained with platelets from hypertensive patients. However sensitivity to higher doses of adrenaline is maintained, both in the infusion experiments and in the tests in vitro

Platelet aggregation after addition of ADP was photometrically evaluated both in standard conditions and after infusion of different amounts of adrenaline (Table V)

In basic conditions, the sensitivity of platelets from hypertensive patients to ADP added in tro (1 micromole) seems to be higher than the sensitivity of normal platelets This finding needs however to be confirmed in a larger group of patients

After infusion of different doses of adrenaline ADP induced aggregation was generally potentiated in both groups, but in the hypertensive group the lower doses of adrenaline had no potentiating effect on ADP induced aggregation

We tried also to compare by means of a canonic correlation coagulation parameters (blood thromboplastin activity and platelet adhesiveness) and clinical parameters (age duration of hypertension diastolic pressure)

Table IV

In vitro platelet aggregation by adrenaline.

	Adrenaline concentration		
	40 µM	80 µM	160 µM
Controls (20)	38,8 %	48,2 %	48,8 %
Hypertensive patients (20)	36,6 %	36,8 %	46 4 %

Table V

In vitro platelet aggregation by ADP (1 2 µM)

	Adrenaline γ/Kg b.w			
	0	5	10	20
Controls (20)	37 6 %	43,5 %	46,7 %	49 8 %
Hypertensive patients (20)	44,8 %	43 9 %	48,1 %	49 9 %

However no significant correlation was found between the two groups of variables. Operating with single correlations, a significant result was obtained for the correlation between plasma thromboplastin activity and the duration of hypertensive disease The increased platelet stickiness, however was unrelated to age duration of hypertension, and the level of diastolic blood pressure (1)

Apart from any speculation about the nature of the increase in platelet aggregation during hypertension, these data suggest that hypertensive patients might benefit from an anti-adhesive and probably from an antiaggregating treatment. In fact pharmacological prevention of thrombosis in the hypertensive may be hampered by the dangers connected with capillary fragility and the tendency to vascular rupture Anti-coagulant treatments of any degree as well as indirect fibrinolytic prevention may be contraindicated.

Therefore hypertension could be a good field for the evaluation of the effectiveness of antiadhesive and antiaggregating drugs used in the prevention of thrombosis and athero-sclerotic vascular disase Regarding the choice of the anti-adhesive or antiaggregating treatment, one should take into account the fact that the increased platelets stickeness observed in this condition is not accompanied by univocal changes in ADP and in adrenaline induced aggregation.

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DISCUSSION

J VERMYLEN The reduction by indomethacin of the proteinuria of chronic glomerulonephritis has been unequivocally demonstrated by Michielsen et al. (1). As platelet aggregation and fibrin formation have been considered as possible mediators of immunologic injury in the kidney we investigated the possible interference of indomethacin with the coagulation processes (2,3)

1 It was confirmed that indomethacin at therapeutic dosage per os markedly inhibits the platelet release reaction.

2. A marked reduction of the 24 hour urinary excretion of fibrinogen-like material was found during the administration of indomethacin to 12 adults with chronic proliferative glomerulonephritis The decrease in proteinuria was less pronounced The degree of selectivity of the proteinuria was not markedly or reproducibly altered We have suggested that indomethacin may reduce intrarenal fibrin deposition in chronic glomerulonephritis presumably by its effect on platelet aggregation

R GROSS Dr Vermylen do you think that it is an immunological reaction which you influence with your treatment with indomethacin

J VERMYLEN It is indeed possible that antigen-antibody complexes and (or) immunoglobulin covered surfaces in the diseased kidney stimulate platelet aggregation, resulting in disturbed circulation and further glomerular damage Indomethacin may inhibit this localized platelet and fibrin deposition Indomethacin has no effect on the immunological procedure we are using to quantitate fibrinogen-related antigen in urine

R. GROSS Dr Coccheri, do you treat your hypertensive patients with anti-aggregating agents whatever the level of hypertension may be

S COCCHERI In our study we considered hypertensive patients those with not less than 100 mm Hg. diastolic pressure Patients are submitted to anti-aggregation only if the systolic pressure does not exceed 180-200 mm Hg.

G LEONE I would like to present some preliminary results on the possibility of a therapeutical approach with aspirin in diabetics with thrombotic tendency Platelet aggregation induced by ADP noradrenaline and thrombin was studied in 20 diabetic patients using the method of Born Thrombin and noradrenaline-induced aggregation as well as the initial rate of ADP-induced aggregation were found to be normal in all patients. In eight of them, however who showed clinical signs of vasculopathy the appearance of disaggregation with critical amounts of ADP (less than 0,5 μM final concentration) was never observed at 37°C (Figure 1) After aspirin ingestion (two grams) these patients showed a rapid disaggregation following ADP-induced aggregation.

M VERSTRAETE Has someone among you any experience on the use of anti-aggregating agents in primary pulmonary hypertension ?

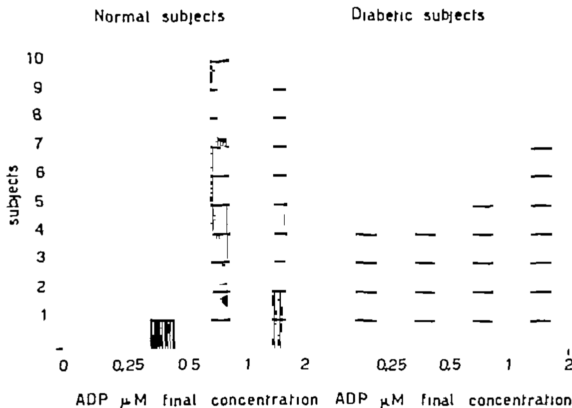


Figure 1

Minimal ADP concentration which induces irreversible aggregation.

R. GROSS That seems to me a very important question, because of the absolute ineffectiveness in my personal experience of anticoagulant therapy in this disease

K. BREDDIN We tried aspirin but we cannot say if it is effective it lowered the aggregating tendency but it did not lower the pulmonary pressure

M VERSTRAETE Dr O Brien, have you some additional remarks on clinical usefulness of pharmacologic inhibition of platelet aggregation ?

J R. O'BRIEN Yes, you should look for drugs that might work in arterial situations and not in venous situations !

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Question N 15

DOES AN IMPAIRED RELEASE REACTION REALLY CAUSE A HAEMORRHAGIC DIATHESIS ?

DISCUSSION

H HOLMSEN
J CAEN

J HUGUES
K. BREDDIN

47 THE NATURE OF THE PLATELET DEFECT IN SOME PATIENTS WITH A BLEEDING TENDENCY

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I would rather invert the question in the sense that some patients with a haemorrhagic diathesis have a severe impairment of the release reaction which may or may not be the whole cause of the haemorrhagic diathesis. On the other hand, not everybody who has an impairment of the release reaction has clinically obvious bleeding, e.g. people who are taking aspirin. It may be that patients with congenital or long-lasting spontaneously occurring defects of the release reaction are recognised as having bleeding problems, simply because they are in this condition for a long time whereas this is not necessarily the case with aspirin eaters. In some of these circumstances, an impairment of release can be a contributory factor other contributory factors may be in the case of aspirin for example the effect on the gastrointestinal mucosa, and in the case of uraemic subjects, there are often abnormalities of the plasma clotting system as well as of the platelets. Having said that I think it is important to try and resolve the apparent differences which some of us have particularly those which I have with Drs ten Cate and Sixma on this point

What do we mean by a normal individual The usual definition is somebody who has not been properly investigated. But I think we should now put this business of second-phase aggregation on a more quantitative basis. We (1) have determined the lowest concentrations of ADP and adrenaline required to produce a second phase of aggregation in 60 normal adults, using the 6-channel aggregometer so that we can get the whole experiment done within 1 2 hours or so All these 60 individuals gave a second phase within a fairly narrow range of threshold concentrations 0.1 1 0 μM adrenaline and 0 1 4 μM ADP

Most of the patients we have studied with bleeding disorders associated with a defect of release have shown no second phase of aggregation with concentrations orders of magnitude greater than this, and the same is sometimes the case after taking aspirin

Another possibility is that the clinical effect of a failure of release may depend not only on the degree of the defect but also on the mechanism underlying it. In some of those patients in whom this condition has arisen spontaneously apparently as a congenital defect, it has been shown by Holmsen and Weiss ( ) and by Mills and Hardisty (3) that there is a severe deficiency of nucleotides in the storage pool of the platelets, which accounts for the failure of release This is associated with a defect of 5HT uptake and storage so that the failure of release of nucleotides is only one aspect of a rather profound platelet abnorma

lity The aspirin group has no such defect of the storage mechanism, and this could perhaps account for some of the apparent differences in clinical severity and also in the bleeding time The patients we have studied with nucleotide storage defects two albinos and several others – have had bleeding times constantly in excess of 20 minutes, which is well out of the range found in people who have taken aspirin

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48. ACQUIRED HAEMORRHAGIC DIATHESIS IN SIDEROBLASTIC ANEMIA (MEGAKARYOCYTOPATHY AND THROMBOCYTOPATHY)

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Paris X France

Our results (3) confirm those obtained by others in platelet const tutional diseases (5 6).

In acquired thrombocytopathies we have done numerous observations in cases of sideroblastic anemias with partial myeloblastosis (1...) frequently followed by myeloblastic leukaemia The patient which we hereby report had 50,000 platelets/ml Ivy bleeding time 6 minutes, decreased platelet factor 3 availability Platelet factor 4 release was also diminished Platelet aggregation estimated either at room temperature or at 37°C gave comparable results i.e defective aggregation using epinephrine and collagen no second wave (at 37°C) w th ADP and a very defective release induced by thrombin Aggregation f washed platelets spontaneously or with ep nephrine was very diminished but it was subnormal using ADP The release was ery defective (4) ADP was rapidly consumed in the plasma, and an increase of plasmatic adenosine deaminase was found with a defective incorporation of C-adenosine (7).

This fact is at variance with Holmsen and Weiss (6) findings in constitutional thrombocytopathies The platelet poor plasma from our patient was an inhibitor of C adenosine in corporation in normal or patient s platelets. The platelet ATP content was in the normal range but platelet ADP was around 40 per cent of the normal Using a modification of the method of Booyse and Ra elson for the study of platelet populations, we (3 8) had found per cent in band C and none in band D (normal range for C + D is around 50 per cent of the total) It seems therefore that the platelets found in the irculation are mostly light and therefore possibly old. Platelet survival time using autologous platelets labelled with radiochromium by N Ardaillou was 6 days

As a matter of fact the megakaryocyte platelet system was disturbed in its whole In the bone marrow we found small and very numerous megakaryocytes (Figure 1) frequently binucleated Using electron microscope we found in these megakaryocytes numerous vacuoles and specially an asynchronism in the formation of granules and demarcating membranes these aspects (Figure) suggest an ineffective megakaryocytopoiesis we also found in the marrow se tions many intracapillary megakaryocytes. In the blood stream, all types of megakaryocytes were easily found with intravascular thrombopoiesis (figure 3). It seems therefore that the platelets found, frequently above the normal range are light and

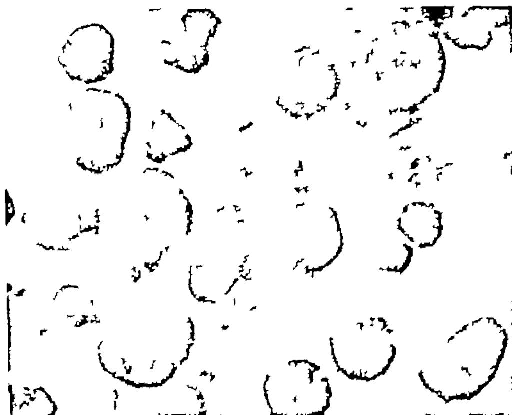


Figure 1

Bone marrow examination (x500) numerous, frequently binucleated megakaryocytes.

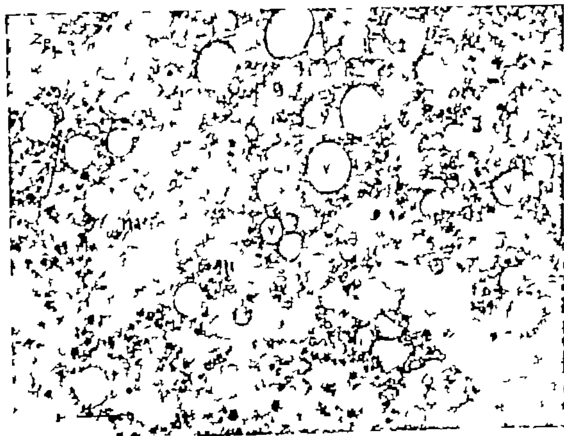


Figure 2

In various areas of the same megakaryocyte disorganized plat let demarcating membranes include only vacuoles (V) or zones including numerous dense granules (G) and demarc ting membranes (Md) Periphe al zone (Zp) Glycogen (Gl) Mitochondria (M)



Figure 3

Cir ulating megakaryocytes (x500) w th platelet formation

old due to the fact the heavy young ones are bound to the circulating megakaryocytes.

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49 HAEMORRHAGIC DIATHESIS IN CHILDREN CAUSED BY ACETYLSALICYLIC ACID AND OTHER ANALGESICS

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Blood loss from the gastro-intestinal tract is a well-known complication of acetylsalicylic acid-therapy

Such bleeding was first attributed to a local irritation (3), but its occurrence after intravenous administration (4), made Weiss (6) to suggest that this haemorrhagic action might be due to an impaired release of platelet ADP

Reviewing our patients with acute intestinal bleeding during the last year we found that three out of four of them presented their symptoms after having taken a normal dose of acetylsalicylic acid for one or two days. The fourth patient, a 9 year old boy developed a severe gastric bleeding after a 24 hour therapy with a normal dose of amidopyrine As far as we know a gastro-intestinal bleeding after ingestion of amidopyrine has not been reported till now but it is not unexpected because we know from the experiments of O'Brien that it is one of the compounds which exerts an inhibitory effect on the platelet aggregation "in vitro" (5).

Chronic gastro-intestinal blood-loss is a common feature in children suffering from juvenile rheumatoid arthritis and treated with acetylsalicylic acid. Reviewing our 4 last cases we observed that two of them presented symptoms of chronic intestinal bleeding with secondary iron-deficient anemia. One of these patients complained about intermittent melaena.

Finally we recently reported three children presenting a non-thrombocytopenic purpura while taking a normal dose of acetylsalicylic acid since a few days (1). Overdosage as well as idiosyncrasy could be excluded. Studies of the platelet function showed an impaired platelet aggregation by inhibition of ADP release We concluded that this mechanism could be responsible for the clinical findings, and that an acetylsalicylic acid therapy should always be considered in the differential diagnosis of a non-thrombocytopenic purpura. Noteworthy is the fact that the father of two of these patients suffered from a severe gastric bleeding on two occasions after a single intake of respectively 2 g and 1 g of acetylsalicylic acid. Two of his children developed purpura while taking the same drug. These phenomena can be independent of each other but they could as well be due to some familial predisposition.

In all the patients in whom coagulation tests and platelet function studies were performed, only an inhibition of ADP release and the concomitant impaired platelet aggregation have been observed. The simultaneous occurrence of a transient haemorrhagic diathesis with acetylsalicylic acid induced platelet dysfunction strongly suggests that these two phenomena are

causally related Nevertheless it remains a fact that only a few children taking acetylsalicylic acid show overt haemorrhagic phenomena, although in all of them the platelet function is disturbed (2) The above mentioned clustering of several cases in two generations of one family makes us wonder whether a possibly genetic factor might not contribute to the appearance of haemorrhagic phenomena.

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DISCUSSION

H. HOLMSEN Dr Caen was the uptake of radioactive adenosine by platelets in PRP measured as counts per minute per ml

J CAEN Yes, it was counts per minute per mL

H HOLMSEN Have you corrected for the platelet number ?

J CAEN Yes, it is the same number of platelets both in saline and in plasma.

H. HOLMSEN In our studies, we found the same uptake per platelet in the patients PRP as in PRP from normal subjects.

J CAEN In the constitutional ones, we have also observed this phenomenon What is peculiar for me is that it seems that the oldest platelets incorporate more adenosine than the younger ones this is completely conflicting with the results in normal human beings.

J HUGUES Did you measure the survival time of these platelets

J CAEN In this case we did not measure the survival time these patients are leukaemic and I am quite sure t is possible to do this.

K. BREDDIN Dr Caen, did you look at the spreading effect in these platelets ? Do the old platelets spread

J CAEN Yes, they spread but more slowly than normal, exactly like in chronic myeloid leukaemia

causally related. Nevertheless it remains a fact that only a few children taking acetylsalicylic acid show overt haemorrhagic phenomena, although in all of them the platelet function is disturbed (2). The above mentioned clustering of several cases in two generations of one family makes us wonder whether a possibly genetic factor might not contribute to the appearance of haemorrhagic phenomena.

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Acta Medica Scandinavica

Supplementum 526

Chronic intravascular coagulation

Clinical spectrum and diagnostic criteria, with special emphasis on metabolism distribution and localization of I^{131} fibrinogen

By

Paul Werner Straub

Distributed by The Almqvist & Wiksell, Periodical Company

Acta Medica Scandinavica

... ...sed as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axe... ...AD ... (from volume 34) this journal was divided into a medical and a surg... section ... (from volume 5) the medical section has been published ... *Acta Medica Scandinavica*.

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ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 526

From the Department of Medicine
(Professor P Frick, Professor A. Labhart)
Kantonsspital University of Zurich Switzerland

CHRONIC INTRAVASCULAR COAGULATION

Clinical spectrum and diagnostic criteria
with special emphasis on metabolism
distribution and localization of I^{131} fibrinogen

BY

Paul Werner Straub

Zürich 1971

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CONTENT

I INTRODUCTION

1 DEVELOPMENT OF THE CONCEPT

Unlike localized coagulation in big vessels, leading to thrombosis, the concept of generalized or disseminated intravascular coagulation has been widely accepted only in the last 10 20 years. The causes, the mechanism, the clinical symptomatology and the *anatomical correlate* are more difficult to understand, though probably not much less investigated. Although the syndrome has been generally recognized only for a short time, the basic experimental observations and conclusions have been made in the last century (233)

1868, Naunyn observed intravascular coagulation of the blood in animals following infusion of hemolyzed erythrocytes (218,219) In 1883 Foà and coworkers made the same observation following injection of organ extracts (107). In 1886 Wooldridge (329), working with tissue extract injections, distinguished an initial "positive phase" with increased coagulability from a second "negative phase with impaired clotting of the blood. This sequence of events was confirmed in 1894 by Martin (191) using injections of viper venom. In 1909 Mellanby (203) attributed the coagulation defect during the phase of retarded coagulability primarily to hypofibrinogenemia. This relation was confirmed by others (129,208). In 1901 DeLee (77), who had observed 3 cases of bleeding in gynecological complications, discussed the possibility of an acquired hemophilia. However only in 1919 Obata (224) correlated the experimental findings with *clinically observed syndromes. He re*lated the symptoms observed after infusion of placental extracts into animals to those observed in human eclampsia, but the results of intravenous infusions of different clot promoting substances were systematically correlated with human diseases only after the second world war The clinical importance of the concept was first fully recognized by obstetricians (113 161,264).

Penick (231) studied the coagulation changes in more detail and stated that thrombocytopenia and a reduction of antihemophilic globulin (factor VIII) are more sensitive indices of intravascular coagulation than hypofibrinogenemia. Experiments with normal and hemophilic dogs, where the presence of hemophilia prevented the thrombocytopenia induced by cold injury (55), suggested that impaired coagulation might protect against intravascular coagulation. This formed the basis for a rational therapeutic approach, the now well established paradoxical therapy of hypofibrinogenemic hemorrhagic states with anticoagulants (251)

Fibrin microthrombi being the anatomical correlate of intravascular coagulation, McKay (201) a pathologist was to collect evidence *for intravascular* agulation as an intermediary

fibrinogen or fibrin (101,205,-86) which are not present in detectable amounts in normal blood, constitutes another indirect argument for the presence of intravascular coagulation. If it is true that fibrinolysis and/or the appearance of fibrinogen/fibrin split products are secondary to intravascular coagulation, both should also be suppressed by diagnostic anticoagulation. We have been able to demonstrate this on four separate occasions with heparin infusions in a patient with prostatic carcinoma and systemic fibrinolysis (?87). On the other hand, inhibitors of fibrinolysis, though suppressing fibrinolysis, should not lead to a normalization of the fibrinogen level if the primary event is consumption by intravascular coagulation unless the secondary fibrinolysis is intense enough to lead to fibrinogenolysis. In the present report, examples for such a diagnostic use of fibrinolysis inhibitors will be presented.

The recent observations, that fibrinogen/fibrin degradation products lead to a prolongation of the thrombin time of plasma (221) and can prevent fibrin monomer from aggregation (5) thus solubilizing fibrin monomers (182) has led to the development of simple methods for the demonstration of soluble fibrin (54,81 1-5) or of complexes of fibrin split products with fibrin monomers (182). Again, these qualitative or semiquantitative methods may yield indirect evidence for a process of intravascular coagulation (54).

Finally intravascular coagulation, acute or chronic, may lead to a peculiar type of hemolysis, characterized by hemoglobinemia, hypohaptoglobinemia and a poikilocytosis of the circulating erythrocytes with triangular helmet and spur forms (so-called burr cells). This hemolysis has been termed "microangiopathic" (49) and may be due to mechanical disruption of erythrocytes during their passage through a fibrin network (60). Since this hemolysis can be suppressed by anticoagulants in vivo and since it is often associated with clinical syndromes where other evidence for intravascular coagulation is found (158) its presence in a given patient has been proposed as an argument for intravascular coagulation (50).

3 AIM OF THE PRESENT STUDY

This report concerns exclusively chronic intravascular coagulation.

1. Twentysix such cases with clearcut chronic intravascular coagulation were investigated in the Department of Medicine Kantonsspital, University of Zurich, from 1964 to 1968 in order to establish the clinical spectrum and the significance of the laboratory signs, including tests for fibrinolysis and the presence of fibrinogen/fibrin degradation products. An unusual case is presented in detail where chronic intravascular coagulation was most probably due to an aortic aneurysm.
2. The results of diagnostic anticoagulation and diagnostic inhibition of fibrinolysis in controversial cases are reported.
3. Turnover and distribi
   I[131] labeled I

undertaken in 33 subjects including normal persons, subjects with obvious chronic intravascular coagulation, and patients with suspected intravascular coagulation, in order to establish the diagnostic value of this procedure.

4 Since the distribution studies with I^{131}-fibrinogen suggested the presence of a "fibrin pool" in many patients, attempts were made in 6 patients to demonstrate a localized accumulation of the labeled fibrinogen in the organism. Previously unreported results obtained with this new procedure include accumulation of radioactivity in a giant hemangioma and in the region of a tuberculous pleura exsudate.

II MATERIAL AND METHODS

1 COAGULATION AND FIBRINOLYSIS

Blood collection and routine laboratory assays for blood coagulation factors in oxalated plasma were performed according to the methods of Duckert (85)

Fibrinogen was determined according to Clauss (67) in many samples also with the heat precipitation method of Schulz (265) and a biuret method on washed clots.

In many patients fibrinogen and factor V were also determined in Trasylol-blood (Trasylol 200 U/ml blood) Thromboelastography was performed according to Hartert s method (136) the assay being done on 0.25 ml of oxalated plasma recalcified with 0 1 ml of 1/10 m calcium chloride

For euglobulin lysis time determinations, fresh oxalated plasma was diluted with 15 volumes of distilled water and a pH of 5.2 was obtained by gentle shaking in a CO_2 atmosphere for three minutes. The precipitate was separated by centrifugation and redissolved in the original plasma volume of buffer Of this solution, 0.5 ml was clotted with 0.05 ml of thrombin (55 u/ml). The lysis time at 37°C was recorded.

The fibrin plate assay was performed according to the method of Astrup and Mullertz (15) using glass slides instead of Petri dishes. Plasminogen was determined using the method of Alkjaersig et al.(6) and the results were reported in per cent of normal plasma.

Immunoelectrophoresis for the detection of fibrin/fibrinogen split products was performed with the method of Scheidegger (259). Dilutions of the reagents were made with 0.9% NaCl solution.

Anti-human-fibrinogen antibodies were prepared in rabbits according to Gitlin (122) The antiserum was absorbed with human serum. It gave no precipitation line when tested against various concentrations of normal human serum The test is sensitive to fibrinogen concentrations not below 5 mg%. Heparin ("Liquemin") was obtained from Roche Basel, Switzerland in ampoules of 5 ml containing 5000 I.U. = 50 mg per ml. ε-amino-caproic acid (Epsylon-Aminocapronsaure") was obtained from Roche Basel, in ampoules of 5 ml, 1 ml containing 400 mg. Trasylol Bayer" 5000 U/ml was used, 1 ampoule containing 5 ml. Bovine thrombin "Roche" was obtained from Roche Basel Switzerland one vial containing 50 NIH U/mg of the dry powder All dilutions, except for immunoelectrophoresis, were prepared in veronal-acetate buffer (Michaelis)

Platelet counts were done according to the method of Feissly Ludin (100)

_. METHODS FOR FIBRINOGEN TURNOVER STUDIES

Studies with fibrinogen I^{131} were carried out in 33 patients hospitalized for various reasons.

a) Isolation and labelling of human fibrinogen

The purification of fibrinogen was performed using the ammonium sulfate precipitation method of McFarlane (194) under sterile conditions. The final precipitate was dissolved in a citrate saline buffer. The clottability of the solution varied between 79 and 96 ?% as determined with a biuret method. For all in vivo studies the same batch of fibrinogen prepared from a fresh citrated plasma pool of 16 healthy blood donors, was used. This preparation, after clarification by high speed centrifugation, and before freezing, had a clottability of 91% and a protein concentration of 10 mg/ml. The clot obtained after addition of thrombin and calcium chloride was not soluble in 5 M urea hence containing fibrin-stabilizing factor and was digested after addition of urokinase hence containing plasminogen. The fibrinogen solution was stored at 0°C throughout the study.

Labelling was performed using the iodine monochloride method of McFarlane (193).

The iodination ratio was less than 0.5 atoms of iodine per molecule of fibrinogen (*assuming a molecular weight of* 300 000). Non-protein iodine was removed with an ion-exchange resin (Dowex 1 x 8 50-100 mesh in chloride form). The free iodine when measured by either trichloroacetic acid precipitation paper chromatography or dialysis, was below 1 per cent and remained so during 14 days storage at 4°C. No precipitate developed and no change in coagulability occurred during the same period of storage. The thrombin clotting time the clot lysis times after addition of Streptokinase (Kabikinase Stockholm, Sweden) and Urokinase (Hoffmann La Roche Basel Switzerland) or plasmin (Kabi, Grade A 66 U/ml) were not influenced by the labelling procedure. Immunoelectrophoresis showed a fibrinogen line not distinguishable from that of normal plasma. The labelled samples were made up to a fibrinogen concentration of 1 ? mg/ml in isotonic phosphate buffer of pH 7.4.

The fibrinogen was injected within 6 36 hours after labelling. All samples were subjected to routine bacteriologic assays and a pyrogen test in rabbits, with negative results. Prior to injection a standard was prepared in duplicate by diluting a weighed 0._ ml of the fibrinogen solution in 1 ml of 20% NaOH and 1 drop of potassium iodide 30% and making up to ?0 ml with distilled water. Two ? ml probes were kept at 4°C as standard.

The patients received Lugol solution 10 drops daily p.o. throughout the study starting 3 days before injection. In some patients iodine saturation was achieved by *injection of 10 ml of Endojodine "Bayer"* 4 hours prior to the fibrinogen injection, followed by daily Lugol solution.

Weighed doses of labelled fibrinogen were injected into the antecubital vein, usually 60 100 μC In 6 experiments where surface scanning or counting was performed, 400 500 μC were injected.

b) Plasma and urine collection

For the collection of heparinized blood at 10, 20 and 30 minutes from an opposite cubital vein, a 10 ml disposabel syringe was rinsed with Liquemin (Roche) prior to blood aspiration.

At 10 minutes and then daily for 8 15 days, 9 ml of blood were collected into 1 ml of an oxalate-Trasylol mixture (sodium oxalate 1/10 m, Trasylol 2000 U/ml).

For plasma volume determination 2 ml of the heparin samples were counted, the values extrapolated to zero time on semilogarithmic paper and compared with the standard.

All Trasylol-oxalate samples, including the mixture of 0.2 ml of the injected labelled fibrinogen solution with 9 8 ml of fresh normal Trasylol-oxalate plasma, were treated as follows:

– ml were used for plasma radioactivity measurement.

– ml were diluted with 1 ml Lysine-ethylester hydrochloride according to Blombäck (4) and 4.5 ml of 0.1 M phosphate buffer containing EDTA (Siegfried, Zofingen) 1 mg/ml and adjusted to pH 5 9 This mixture was clotted with 0.5 ml of thrombin 700 U/ml (Roche Basel) and left at room temperature for hours. The clot was collected on a silk cloth, washed and dissolved in ml of alkaline urea (42) After counting, fibrinogen was determined using a biuret method.

– 2 ml of plasma were mixed with 2 ml of 10 per cent trichloroacetic acid and left at 4°C for 1 hour. The precipitate was removed by centrifugation and 2 ml of the supernatant were counted.

24 hours-urine portions were collected for 8 9 days. After thorough mixing, 5 ml were removed and the radioactivity concentrated according to the method of Iber (151).

Radioactivity of all 2 ml samples and of the concentrated urine specimens was measured simultaneously at the end of the study of each patient in a well-type scintillation counter (Picker Twinscaler II) with automatic background subtraction. Correction for decay was done by reference to the standard.

c) Calculation of plasma half-life time, fractional catabolic rate and distribution of fibrinogen

The model elaborated by Pearson, Veall and Vetter (230) for albumin was used. It combines the equilibrium time method of Campbell et al.(61) with the renal clearance technique of Berson and Yalow (34) and is based on the assumption that the behaviour of labelled fibrinogen corresponds to an open two compartment (mamillary) system, that I^{131} is rapidly excreted when the labelled molecule is catabolized and that catabolism is exclusively taking place in or in close connection to the intravascular compartment the extravascular compartment serving only as a r

servoir. In a steady state, the rate of metabolic degradation is equal to the rate of synthesis.

The radioactivities of all plasma samples were expressed as percentage of the total radioactivity at zero time. The latter was obtained by extrapolation using the values at 10, 20 and 30 minutes. Fig. 6,7 show the graphical expression of the data on a semilogarithmic scale. After 2-3 days, the disappearance of radioactivity from plasma becomes linear. The values form day 3 to the end of the study were analyzed by the method of least squares using a table computer "Olivetti Programma 101" and were closely described by an exponential equation $x = Ce^{-bt}$ where C is the intercept of the slow and linear component of the curve with the ordinate at zero time and b corresponds to the slope of the curve. The variance s^2 is a measure of the fit of the mathematically obtained curve with the experimental data (297).

The radioactivity excreted in the urine during each 24 hours-period was expressed as a percentage of the injected dose. When these figures were successively subtracted from 100% a curve was obtained describing the residual radioactivity in the organism at each day. When plotted on semilogarithmic paper again a linear disappearance curve was obtained but without the initial rapid component. These data, starting from day 1 up to day 8 were also analyzed by the method of least squares and were well described by the simple exponential function cited above. The percentage of radioactivity outside the circulating blood was obtained for each day by subtraction of the plasma radioactivity from the total remaining activity.

The fractional catabolic rate was obtained by dividing the percentage of the dose excreted in the urine during a given day by the average percentage of the dose actually in the plasma at the same day and calculation of the average of this figure for the whole study.

The distribution of fibrinogen in the organism was calculated in relation to the total plasma fibrinogen pool assuming that the specific activity of the fibrinogen in plasma is equal to that of the total fibrinogen in the body (230). The extravascular fibrinogen as calculated by subtraction of the plasma activity from the total remaining activity reaches a maximum after a few days and then declines at an exponential rate similar to that of the plasma curve. When this maximum is reached (equilibrium time s.fig.6) plasma and extravascular radioactivity are in a transient equilibrium, the amount of labelled fibrinogen leaving the intravascular space being equal to that returning from the extravascular compartment. The figures for plasma (Q_P) and extravascular (Q_E) percentages of radioactivity at equilibrium time indicate the relative sizes of the two pools. Since the total plasma fibrinogen pool can be calculated from plasma volume and plasma fibrinogen concentration, the total body fibrinogen pool is given by the formula

total body fibrinogen = plasma fibrinogen pool $x = \frac{Qp + Qe}{Qp}$

Because in many patients the curves for plasma and total remaining activity though both linear were not parallel, e.g. less radioactivity appeared in the urine than would have been expected from the plasma decay, the distribution was also

calculated according to Amris (10). In pathological states, even in the steady state the plasma disappearance may depend on degradation and on conversion of fibrinogen to fibrin. These authors therefore postulated a "fibrin pool". The ratio $\frac{Q_P + Q_E}{Q_P}$ then indicates the ratio between the sum of the total fibrinogen plus "fibrin" accumulated from zero time, and intravascular fibrinogen. When the percentage of intravascular fibrinogen in relation to the total remaining activity $\frac{Q_P \times 100}{Q_R}$ is calculated for each day a distribution curve is obtained. When the ratio is constant, e.g. the plasma and the total remaining curve are parallel, the slope of the distribution curve is 0. When the two curves are divergent, the ratio of intravascular to total remaining activity decreases and the distribution curve is no more horizontal. The intercept of the distribution curve with the ordinate gives the actual percentage of intravascular fibrinogen. The "fibrin pool" can be calculated by subtraction of the total fibrinogen obtained with the distribution curve, from the total fibrinogen plus "fibrin" calculated at equilibrium time.

d) Surface radioactivity

Surface radioactivity was measured using a nucleoscope "Siemens" with a 25 mm lead shielding and a background of 160-180 c.p.m. The counts were corrected for background and radioactive decay. The relative amount of radioactivity over a particular organ in comparison with the activity measured over the heart was calculated each day in $\frac{\text{c.p.m. organ}}{\text{c.p.m. heart}}$

III. RESULTS

1 LABORATORY FINDINGS IN 26 PATIENTS WITH ACQUIRED HYPOFIBRINOGENEMIA MOST PROBABLY DUE TO CHRONIC INTRAVASCULAR COAGULATION

In this series (table 1) only those patients were included who had an acquired hypofibrinogenemia of less than 150 mg% according to the method of Clauss. In fact, in 25 of the 26 patients, the value was 100 mg% or below. Patients with chronic primary fibrinolysis, as observed in cirrhosis of the liver were excluded.

a The *underlying disease* was malignant neoplasia in 7 patients 17 patients had a carcinoma, all of them generalized. In 7 patients, the carcinoma was prostatic. This group is responsible for the over all prevalence of males over females. In the other patients, both sexes were equally affected. The patients with carcinoma and a mean age of 69 years (45 84) also account for the high over-all mean age of 5 years. 10 patients with a mean age of 40,5 years (14 64) had acute leukemia, with a striking prevalence of the rare promyelocytic variant of leukemia. Finally 3 patients had a giant hemangioma and another patient with aortic aneurysm, showed no evidence of neoplastic disease at autopsy. This case will be discussed in more detail.

a) Analysis of coagulation

The assays were performed within 10 60 minutes after blood collection in most patients. A moderate to severe reduction of the Quick value was found in all patients (table 1). The recalcification time was markedly prolonged and the prothrombin consumption decreased in some but not all patients. *Fibrinogen measured* with Clauss thrombin clotting time method was consistently reduced (0 125 mg%). In 6 of 7 patients where blood was also collected with addition of a fibrinolysis inhibitor the respective value for fibrinogen was not significantly different from that obtained without inhibitor. In 13 cases the value was also in good agreement (± 30 mg%) with that obtained using the heat precipitation method of Schulz. The latter method which also measures incoagulable derivatives of fibrinogen and fibrin, gave higher values in 9 a lower value in only 1 patient.

Prothrombin (29 100%) was found below the normal range in only half the patients. *Factor V* (15 100%) was below 50% in 16 of the 26 patients. In only one of 7 cases, where the assay was also done on Trasylol blood was the value significantly higher than in blood obtained with-

Tab. Data on blood coagulation and fibrinolysis in 26 patients with defibrination syndrome most probably due to disseminated intravascular coagulation

Patient	Age Sex	Diagnosis	Quick %	[illegible]	[illegible]	Fibrinogen mg%		Factor II %	Factor V %	Factor VII-C %	Factor VIII %	Factor IX %	Factor X %	[illegible]	Platelets per mm³	Lysis in TEG	[illegible]	[illegible] %	F.D.P. Plasma	F.D.P. Serum
M.R.	58 m	Ca. prostate	37	100	<2	45	20	60	31	73			43	34			35	100		
F.W.	64 m	Ca. prostate	57	115	<3	60	60	100	36	100	90	36	34	30	93000	1.5	80	5		
B.R.	61 m	Ca. prostate	40	92	<3	30	100	82	29	100			74	23						
K.A.	54 m	Ca. prostate	36	110	37	35(30)	8	70	90(92)	32	100		46	20	19000	>4	30	17		
T.R.	74 m	Ca. prostate	44	103	30	84(80)	80	32	64(70)	80	74	100	43	21	93000	>4	8	30		
R.K.	73 m	Ca. prostate	38	103	21	125(115)	120	70	43	80	36		80	16	3000	<3	4	64		
Ch.G.	69 m	Ca. prostate	32	120	46	80	90	96	100	86	6		62	34	31000	<1	40	18		
T.A.	64 f	Ca. breast	47	94	<4	45	30	30	80	64	5		54	30	52000	>4	8			
W.A.	63 f	Ca. breast	44			43		>100	41	>100			78	31	185000	>4	8	67		
R.D.	70 m	Ca. stomach	36	∞	3	30	60	45	40	100	30	74	70	23	164000			32		
W.M.	47 f	Ca. pancreas	33	82	<2	17	100	29	16	64	31	100	50	40		>6	0	51		
O.E.	43 m	Ca. liver	32	140	<3	35		30	32	43	16	33	38	19	73000			43		
A.I.	44 f	Promyel. Leukemia	48	105	<3	85(70)	95	41	40(44)	43	17	76	34	18	23000	>4	8	27		
M.A.	34 m	Promyel. Leukemia	40	126		8	58	100	80	40	100	78	45	20	36000	>4	8			
Z.H.	33 f	Promyel. Leukemia	38	92	45	80(>140)	140	95	40(72)	78	200	>100	74	18	14000	>4	8			
F.E.	13 f	Promyel. Leukemia	8	>400	100	8	6	82	15	96			100	>120	18000		8			
D.F.	22 m	Promyel. Leukemia	38	178	30	43	34	74	42	58	100	100	38	34	14000	>4	8			
W.L.	36 f	Promyel. Leukemia	41	123	80	78	170	90	48	33		100	76	17	25000					
D.I.	35 f	Promyel. Leukemia	41	85	16	65	168	43	16	74			37	18	17000	>4				
M.K.	38 f	Promyel. Leukemia	15	95	6	27	30	32	15	18	1200		18	34	76000		80			
S.H.	64 f	Myeloid. Leukemia	44	118	19	80	163	30	45	30			25	26	90000					
G.M.	64 m	Monocyt. Leukemia	27	173	7	100	140	54	20	18			31	30	6000	>4				
R.T.	30 f	Giant Haemangioma	60	83	4	80(74)	<120	52	>100(>400)	62				21	217000	>4		43		
L.A.	1/4 f	Giant Haemangioma	65	80	3	300	170	40	92	64			66	23	38000	>4				
T.R.	4 f	Giant Haemangioma	43	120	23	82		92	90	60			34	27		>4	8			
R.P.	87 f	Aortic Aneurysm	41	150	26	30(20)	< 50	45	39(30)	>100			96	24	70000	>4	0			
NORMAL VALUES			70 100	90 120	1-7	150 300	180 -400	70 -400	80 180	70 -100	50 -150	80 150	70 100	13 16	150000 -400000	4	0	70 -100		

Values in brackets: Fibrinogen acc. to Clauss and Factor V determined in Eurypivi plasma

VII-C VII-Complex

F.D.P. Fibrinogen/Fibrin Degradation Products

out inhibitor addition. In this sample (patient Z.H.) the fibrinogen value showed the same discrepancy which was most probably due to the fact that this patient's blood was assayed several hours after blood collection. *Factor VII Complex* was reduced in 14 of the 26 patients. *Factor VIII* showed wide variations (5 1–80%) in the 14 patients where this assay was performed. *Factor IX* was abnormally low in 2 of 9 cases. *Factor X* was reduced in 16 of 25 cases.

The thrombin time (normal 13 18 seconds) was prolonged in 21 of the 26 cases. In 19 of these 21 fibrinogen degradation products were assayed, with a positive result in 12. Of the remaining 5 cases without prolongation of the thrombin time 3 had demonstrable fibrinogen degradation products, one had none and in one the assay was omitted. Thus, a prolonged thrombin time did not necessarily indicate the presence of fibrinogen degradation products and vice versa. *Platelets* were above 150 000/mm^3 in only 2 patients. They were usually reduced quite drastically as indicated by the mean value of 58 000

Fibrinolysis tests were done in 21 cases and found consistently negative in 15 of them. In an additional patient, only a thrombo-elastogram was done which showed no plasma clot lysis. All 6 patients with positive direct tests for fibrinolytic activity (euglobulin lysis time or fibrin plate or both) also showed fibrinogen degradation products. 5 of these 6 had prostatic carcinoma. Nevertheless, 5 of the 15 patients with negative fibrinolysis tests also had demonstrable fibrinogen degradation products. Plasminogen was determined in 12 patients and showed wide variations. Of the 7 patients with values below 50% only 3 had positive tests for fibrinolysis, while 6 of the 7 had a positive test for fibrinogen degradation products. Finally in 24 of the 26 patients immunoelectrophoresis was done using specific antifibrin antibodies for the detection of *fibrinogen/fibrin degradation products*. The test was positive in 15 patients and was therefore much more useful in this condition than the most sensitive direct test for systemic fibrinolysis, the euglobulin lysis time method. In only 3 cases the blood was assayed for the presence of cryoproteins. In all 3 a plasma cold precipitate (most likely cryofibrinogen) was found in the absence of a serum precipitate

b) Fibrin thrombi

The only really direct evidence for disseminated intravascular coagulation is the histological demonstration of fresh fibrin microthrombi in the microvasculature 20 of our patients died. Autopsy reports were available in 14 of them. Fibrin thrombi were reported in none. But it has to be noted, that no fibrin stains were employed except in 5 patients with leukemia and that no systematic search for thrombous was done. Adequate search for fibrin thrombi in the 5 patients with leukemia was negative.

c) Diagnostic in vivo fibrinolysis inhibitors

4 patients were tre[illegible] amino caproic acid one [illegible] a primary

[illegible]

In patient G.G. (Fig. 2) [illegible] ε-ACA [illegible] did not increase the fibrinogen [illegible] days [illegible] the [illegible] disappearance of [illegible]. Only after a few [illegible] therapy [illegible] gradually [illegible] but since the [illegible] after discontinuation of ε-ACA the fibrinogen remained [illegible], it must be assumed that the [illegible] process had stopped spontaneously. This patient [illegible] and is still living after 5 [illegible] on estrogen therapy.

In the third patient (T.A.) who had a generalized breast cancer, no change in the fibrinogen level was observed after [illegible] days of ε-ACA therapy (Fig. 3). At autopsy this patient had diffuse liver metastases and fatty transformation of the residual liver tissue.

The fourth patient (Z.H.) had [illegible] cytic leukemia. In this patient the liver was not enlarged [illegible] phosphatase and cholesterol were normal. At autopsy the liver was grossly normal but histologically had leukemic infiltrates.

[illegible]

was negative for bile pigment. Serum bilirubin was 0,2 mg%.

On December 22 1967 the patient had an attack of severe pain in the

Patient I.H. 74 yrs. Prostatic Carcinoma

Fibrinogen 1g 

E-ACA gr/day  

Fibrinogen Turnover
Plasma t/2 1.5 days

Fibrinogen mg per 100ml
method of Clauss
method of Schulz

Factor V %

Factor X %

Thrombin time sec

Euglobulin lysis time hrs 4 >4 >4 >4 4 >4 >4 >4 >4 >4 >3 >4 >4 >4 >4 >4

Fibrin plate

FDP Plasma / Serum

Plasminogen % 0 36 17 47 63

Fig. 1 Coagulation and fibrinolysis results in a patient with prostatic carcinoma. Despite therapy with -amino caproic acid, the plasma-half-life time of labelled fibrinogen was reduced to 1.5 days.

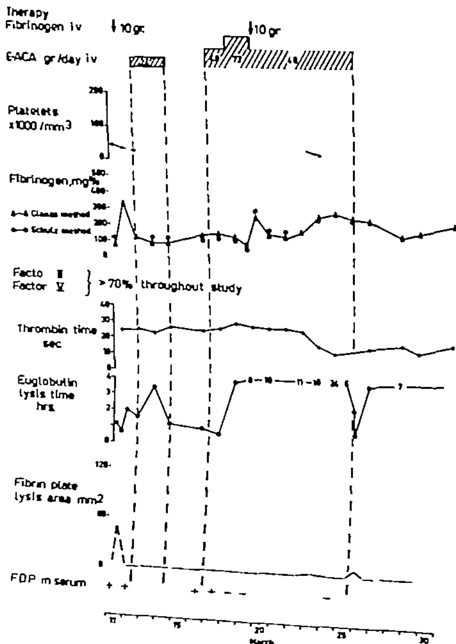
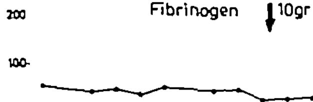
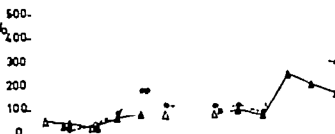


Fig. 2: Coagulation and fibrinolysis results in a patient with carcinoma of the prostate. Even excessive doses of -amino caproic acid, although suppressing systemic fibrinolysis, did not lead to an increase of the fibrinogen level. Further comment s. results.











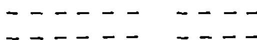


Fig. 3: Coagulation and fibrinolysis results in a patient with carcinoma of breast and chronic hypofibrinogenemia. While on heparin therapy a slight increase of the fibrinogen level was observed. The association of amino caproic acid had no apparent effect.

right upper abdomen, the right chest and the back, a rapidly developing left pleural effusion and a generalized bleeding tendency. She died December 26 following another attack of severe pain.

At autopsy a ruptured aneurysm of the descending aorta was found with bilateral haemothorax, mesaortitis "en plaques" with extensive parietal thrombi in many layers. The liver weighed 620 gr and demonstrated a posthepatitic cirrhosis. The spleen weighed 160 gr. No metastases of the breast carcinoma were found.

This patient had thrombocytopenia, severe hypofibrinogenemia with depression of factors II and V in the presence of normal factors VII and X and in the absence of demonstrable fibrinolysis. The prolongation of the thrombin time was due to the hypofibrinogenemia as shown by the normalization following fibrinogen infusion. No active fibrinolysis and no fibrinogen degradation products appeared following fibrinogen infusion. Although Trasylol, infused following administration of 4 gr fibrinogen, did not prevent a drop of fibrinogen to a level lower than expected, the duration of therapy was not sufficient to definitely judge on the effect.

d) Diagnostic use of Heparin therapy

The results of Heparin therapy in 4 of the patients with promyelocytic leukemia have been invalidated by concomitant extensive bleeding, multiple transfusions or premature discontinuation of heparin because of hemorrhage (..89). In patient P.W. with prostatic carcinoma, heparin infusions during 4 periods completely suppressed the systemic fibrinolysis (287) thus suggesting that fibrinolysis was secondary to intravascular coagulation. In patient T.A. with breast cancer (fig. 3) fibrinolysis was not present. The fibrinogen level showed only a slight rise during adequate heparin therapy and no complete normalization occurred. After infusion of 10 gr of fibrinogen while on heparin therapy the drop of the fibrinogen level was not greater than expected when the equilibration with the extravascular space is taken into account.

e) Comment

During these chronic states of defibrination, the impressive clinical findings associated with acute intravascular coagulation, such as shock, cyanosis, oliguria etc., are absent and a characteristic pattern of laboratory results would be of critical importance. From the above results, however, no definite coagulation pattern emerges which could be generalized as typical for chronic intravascular coagulation. Since low fibrinogen values were used as a criterion for inclusion in the study, hypofibrinogenemia is the common denominator, but the wide variation and lack of correlation of the other values suggests that many cases of intravascular coagulation may be missed when hypofibrinogenemia is a mandatory finding for establishing the diagnosis.

Nevertheless, for practical purposes it is important to note that all patients had a reduced Quick value. Chronic intravasc//

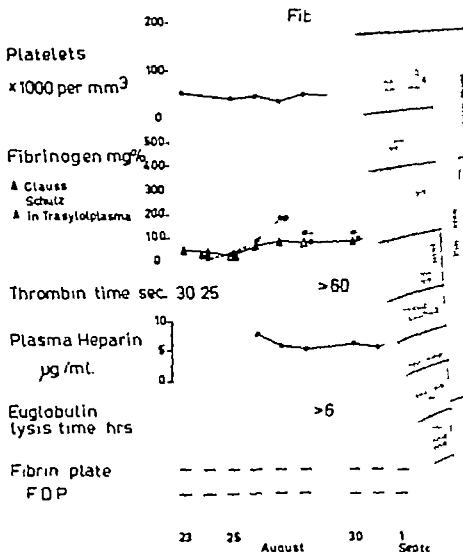


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lar coagulation must therefore be included in the list of the causes of a low Quick. The suspicion is greatly intensified when thrombocytopenia is present especially since most other causes of thrombocytopenia except hypersplenism in liver cirrhosis are not associated with a low Quick value The diagnostic value of thrombocytopenia is unfortunately reduced by the fact that bone marrow invasion may be responsible for it in cases with malignant diseases. The fibrinolysis test may be negative or positive but fibrinogen degradation products are found in a considerable number of patients. The fact that fibrinogen degradation products may be found despite absence of direct evidence for systemic fibrinolysis, may be attributed to insufficient sensitivity of the direct fibrinolysis tests to an episodic nature of the fibrinolytic process or to strictly localized proteolysis of the deposited fibrin (.86)

It also appears that factors II (prothrombin) and V which are expected to be consumed in intravascular coagulation may be normal despite severe hypofibrinogenemia. The level of the other clotting factors is also not of much value since factors VII IX and X may be affected by concomitant liver disease often present in patients with generalized malignant disease and since factor VIII may be consumed or activated by both coagulation and fibrinolysis.

When the patient groups are compared, it appears that 5 of 6 patients with positive tests for fibrinolysis had prostatic carcinoma. In the other diseases a positive fibrinolysis test was rare

In patients with demonstrable increase of systemic fibrinolysis, the differentiation from primary fibrinolysis may be difficult especially because in cases with generalized prostatic carcinoma the almost mandatory bone involvement may be responsible for the thrombocytopenia. In doubtful cases, the diagnostic use of fibrinolysis inhibitors or anticoagulants may be helpful.

Only three patients had an adequate trial of a fibrinolysis inhibitor In all three fibrinolysis was suppressed. In the first the fibrinogen level was not affected in the second 8 gr of infused fibrinogen did not raise the fibrinogen level nor was its drop prevented during the subsequent 24 hours. In the third patient even excessive doses of ε ACA though suppressing systemic fibrinolysis did not raise the fibrinogen level during 6 days. Thus, ε ACA did not stop the defibrination process as would be expected in cases with primary fibrinolysis, even though fibrinolysis was rapidly and effectively suppressed This presents indirect evidence for the view that the primary event in these patients is other than fibrinolytic.

If intravascular coagulation were going on in these patients heparin therapy would be supposed to suppress the precess and thus induce a normalization of the clotting factors Moreo er if the occasionally observed systemic fibrinolysis were secondary to intravascular coagulation fibrinolysis should also be suppressed by heparin. In one patient with prostatic carcinoma we could conclusively demonstrate the latter effect of heparin on 4 occasions (.87). However the above results do not give conclusive data as to the first effect of heparin namely normalization of the fibrinogen level.

[illegible] OF [illegible] FIBRINOGEN STUDIES IN [illegible] PATIENTS

The patients submitted to [illegible]-fibrinogen turnover studies have been grouped according to their underlying disease (table 4). In group 1 no demonstrable disease was present at the time the study was performed. In group 2, 5 patients had arteriosclerosis and at the time of the study were recovering from vascular complications thereof. An additional patient had malignant hypertension, another a generalized scleroderma. All cases in group 3 had active inflammatory processes. The patients of group 4 had advanced neoplastic disease of diverse origin. The 3 last patients of this group had demonstrable chronic intravascular coagulation and are included also in table 1 of the preceding section. Finally in group 5 5 patients with miscellaneous diseases are found.

During the turnover study all patients were in a steady state as demonstrated by a constant plasma fibrinogen level.

a) Tracer data from plasma and urine

The graphical expression of the tracer data is illustrated by figures 6 and 7 The values for the radioactivity in plasma, that retained in the body that in the extravascular space and that daily excreted in the urine are all expressed as percentage of the injected dose The 100% value was obtained by extrapolating the plasma values of the 10 20 and 30 minutes samples to zero time The mathematical results are given in tables 2 6 In 9 patients no urine data are given because of inadequate urine collection (cf. figures 8 [illegible]). Examples are presented of results of the various groups of underlying diseases.

– Plasma disappearance

When plotted on a semilogarithmic scale after 2 3 days of equilibration with the extravascular space the disappearance of the plasma radioactivity was described by a straight line in all cases. Although in most patients the value of day [illegible] was already within the statistical limits of the straight line, only the values from day 3 onwards (average 7 points, range 4-11) were used for calculation of the plasma curve. The variance s of the slope b, which is a measure of how well the experimental points are fitted by the straight line was very low in all cases. It amounted to 0.0005 (0.0001-0.0010) in group 1 0.0006 (0.0001-0.0011) in group 2, 0.0011 (0.0003-0.0034) in group 3 0.0014 (0.0001-0.005) in group 4 and 0.0015 (0.0003-0.0035) in groups 5 and 6

The plasma t/2 was 3.9 (3.6-4.5) days in the control individuals. The group with vascular diseases (arteriosclerosis malignant hypertension scleroderma) had a mean t/2 of 3.? (2.8-3.6) days, when the 3 patients on oral anticoagulant were not included The latter had [illegible] t/2 of 3.8 (3.2-4.5) days.

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Table 3 Results of fibrinogen turnover studies in patients with arteriosclerosis, malignant hypertension, scleroderma

	Initial	Record number	Age	Sex	Diagnosis	Weight kg	Sed rate mm/h	Hct %	Quick %	Platelet count per mm³	Plasma Fibrinogen mg/ml	Plasma Volume ml/kg	Total Plasma Fibrinogen mg/kg
Arteriosclerotic subjects	R.M.	1644.67	74	f	Hypertension, Stroke	49.7	8	41	96	normal	3.6	34.3	123
	M.H.	9.68	77	m	Hypertension, Stroke	92.0	35	35	73	325000	3.4	31.5	107
	Z.A.	1820.67	73	f	Hypertension, myocardial infarction	61.8	25	40	85	326000	4.0	31.9	128
	S.W.	1415/67	58	m	Myocardial infarction	66.4	21	43	100*	normal	3.3	38.4	127
	W.I.	2097.67	69	f	Hypertension, Stroke	65.3	45	34	67*	normal	4.1	32.2	132
	R.E.	2150.67	61	f	Hypertension, Stroke	65.0	14	38	96*	normal	2.6	44.9	117
	C.M.	3225.68	43	m	Malignant hypertension	84.5	31	40	73	517000	5.2	34.9	182
	K.D.	3595.68	39	f	Generalized scleroderma	58.0	16	35	~100	194000	3.2	42.2	135

Initial	Data obtained from plasma curve: Plasma t/2 days	λ of slope	Extrapol. plasma activity at t=0 %	Turnover rate: mean catabolic rate from plasma and ... data	mg/kg/day	Pool size: from data curve % intravascular	TEF mg/kg	from ... % intravascular	TIF mg/kg	Extra-Pool TEF − TIF mg/kg
R.M.	2.8	0.0101	55.3	42.6	52.3	73.3	163	76	166	6
M.H.	3.3	0.0111	[illegible]	31.2	33.6	71.3	[illegible]	[illegible]	151	41
Z.A.	3.6	0.0103	51.5							
S.W.	4.5	0.0107	5 3	22.9	29.1	63.3	167	67	157	22
W.I.	3.2	0.0101	53.3	34.7	45.8	74.5	177	[illegible]	174	6
R.E.	3.8	0.0089	53.9	31.6	36.3	64.4	182	61	13	9
C.M.	3.4	0.0107	45.6							
K.D.	3.6	0.0102	[illegible]	35.6	4 3	63.9	175	77	5	6

* These patients were on oral anticoagulants throughout the study. The Quick value is that of admission.

Table 6 Results of fibrinogen turnover studies in miscellaneous diseases

Group 5 Miscellaneous

Initial	Record number	Age	Sex	Diagnosis	Wght. kg	Sed. rate mm/h	Hct. %	Quick %	Platelet count per mm^3	Plasma Fibrinogen mg/ml	Plasma Volume ml/kg	Total Plasma Fibrinogen mg/kg
H.U	328/68	18	f	Acute glomerulonephritis	63.5	14	25	68	217000	3.9	45.6	178
B.B.	676/68	29	f	Kidney transplant	56.6	7	29	100	190000	1.6	43.1	69
Z.W	627/68	43	m	Kidney transplant	58.5	7	33	100	112000	1.2	40.9	49
M.C.	151/68	36	m	Cirrhosis of liver	58.2	27	37	40	90000	2.1	41.5	87
R.K.1	3196/68	30	m	Congenital cyanotic heart disease (Ebstein anomaly), erythrocytosis *1st study* no therapy	53.0	1	72	90	131000	1.6		
R.K.2	3592/68	30	m	2nd study heparin infusion	56.1	1	69	70	113000	2.3	35.5	81

Initial	Data obtained from plasma curve			Turnover rate		Pool size				
	Plasma t/2 days	s^2 of slope	Extrapol. plasma activity t1=0,%	mean catabolic rate from plasma and urine dat %/day	mg/kg/day	from distrib. curve % intra-vascular	TLF mg/kg	from relation at equilibrium time % intra-vascular	TFF mg/kg	*Fibrin-Pool TFF − TLF mg/kg
H.U	2.9	0.0004	54.3	39.2	70.0	60.9	292	50	356	64
B.B.	1.7 (1.4)	0.0035	87.1	59.6	41.1	90.1	77	69	100	23
Z.W	1.6 (1.3)	0.0009	62.1	89.0	43.6	48.2	102	56	88	–
	3.3	0.0003	58.5							
	3 1)	0.0013	33.4							
	2	0.0027	43.2	39.0	31.6	65.0	124	47	172	48

Note: the corresponding values for t/2 of isolated fibrin

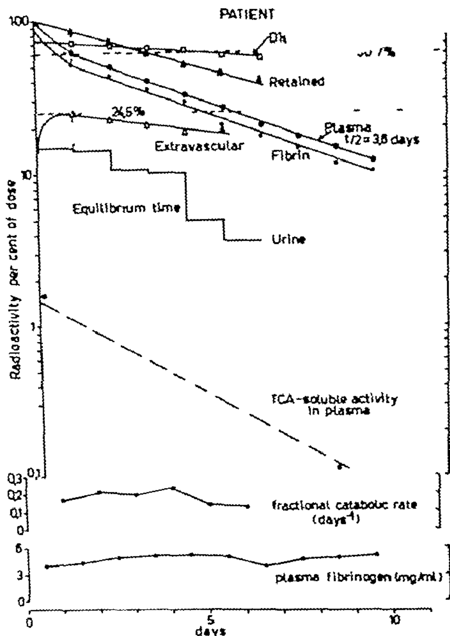


Fig. 6 Graphical expression of the results of fibrinogen turnover and distribution studies in a patient of the control group. For explanations s. methods and results.

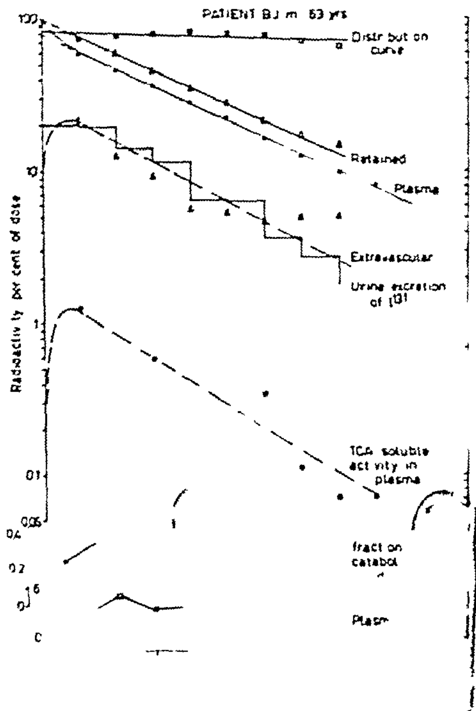


Fig. 7 Gr... in this patient w... ar parallel and th... (... methods and ...

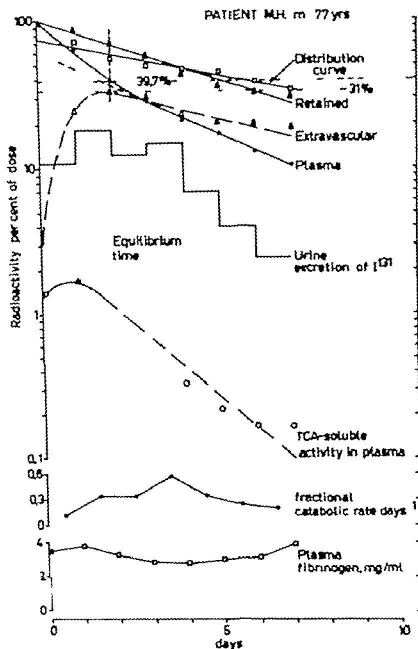


Fig. 8. Results of fibrinogen turnover and distribution study in a patient with hypertension, arteriosclerosis and encephalomalacia. Plasma t/2 was 3.3 days.

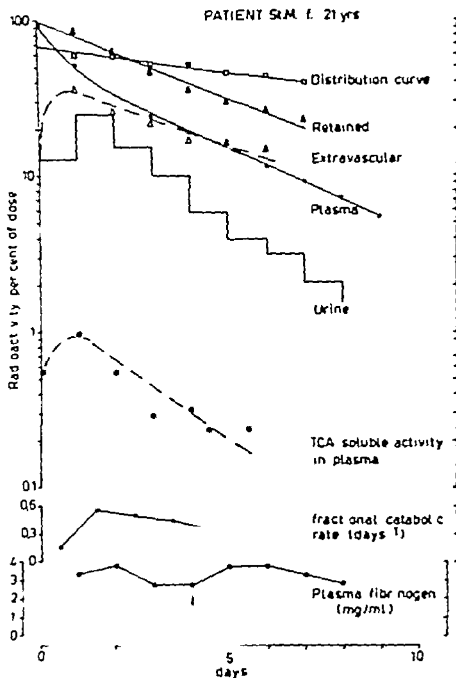


Fig. 9 Fibrinogen turnover studies in a girl with severe paralysis due to polyradiculitis. Plasma t/2 I-131-fibrinogen was 3.2 days.

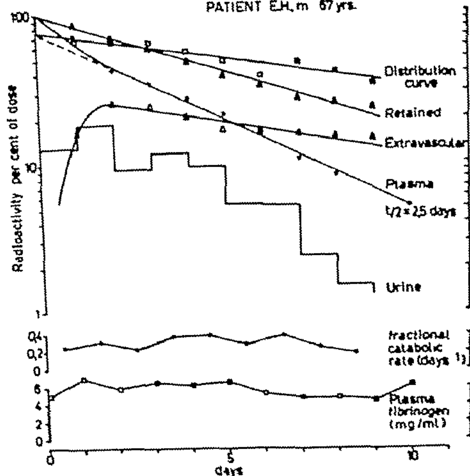


Fig. 10. Results of the fibrinogen turnover study in a patient with generalized carcinoma, probably lung cancer, in whom the coagulation analysis did not show evidence for intravascular coagulation. Nevertheless, the plasma t/2 of I-131 fibrinogen was shortened and there was a marked divergence of the curves for plasma and retained activity suggesting fibrin deposition.

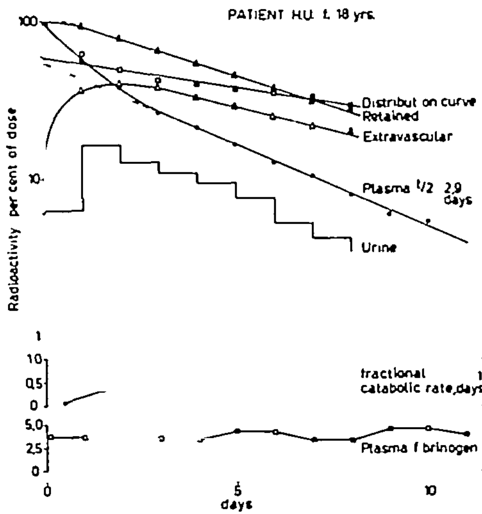


Fig. 11 Results of I^{131}-fibrinogen studies in a patient with acute glomerulonephritis. The plasma t/2 was shortened, the fractional catabolic rate increased to a mean of 39.2% per day

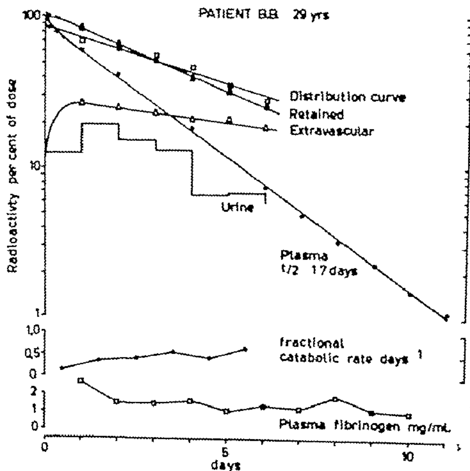


Fig. 12. Fibrinogen I^{131}-study in patient with kidney transplant during a phase of chronic rejection of the transplant.

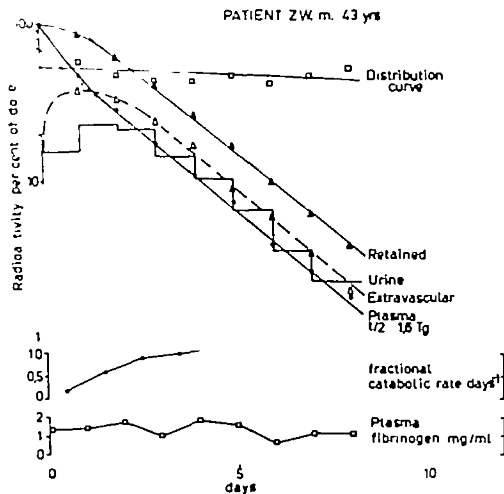


Fig. 13. Fibrinogen I[131]-study in tiss lant, studied dur beginning transplant rejection.

patients with inflammatory processes was 3.3 (_5-4 1) days. Both patients with tuberculosis had a definitely shortened half-life (_9 and _5 days) The group with neoplastic diseases including the patient with giant hemangioma, had an average t/2 of 2.1 (1.5-_5) days. All patients of group 5 had a t/2 below 3.3 days, with the most marked shortening (1 7 and 1.6 days) in the 2 patients with kidney transplants.

Since daily determinations of coagulability were done on the plasma samples, decay curves could also be established for radioactive fibrin (clottable fibrinogen) in all patients (fig. 6). In tables 6 t/2 for fibrin is only given when it differed from that of the whole plasma curve, e g. in those patients where the clottability of the radioactivity in plasma decreased during the study (fig. 14) Such a decrease was only observed in 5 patients with neoplastic diseases, particularly in all 3 with chronic intravascular coagulation, in both patients with kidney transplant and finally in the patient with congenital cyanotic heart disease

Cumulative urinary excretion of the radioactivity

The percentage of the injected dose eliminated after 24 hours, 4 days, 6 days and 8 days is given in table 7 for the different patient groups. In the "normal" individuals 14.6 (10 7 17.3) % was eliminated after 24 hours and 68.1 (65 1 74.6) % was excreted at day 8 In the other groups, elimination was more rapid.

When the percentage of the initially injected radioactivity excreted in the urine during successive _4 hours periods, was cumulatively subtracted from 100% a curve could be drawn for the total radioactivity retained in the body at each day Again, this decay followed a straight line. It was parallel with the plasma disappearance curve in 13 patients. It was divergent in the rest of the patients, where the slope of the curve for the retained activity was less than that of the plasma activity Thus, in many patients, despite adequate collection,less radioactivity appeared in the urine than would have been expected from the plasma disappearance curve This divergence of the curves was in no apparent correlation with the underlying disease except that parallelity of the curves was never seen in the patients with neoplastic disease

Extravascular radioactivity curve

When the radioactivity in the plasma was subtracted each day from the total activity retained in the body the difference indicated the amount of activity outside the circulating blood. The term extravascular radioactivity could however be misleading, since this fraction may include fibrin, deposited intra- or extravascularly This extravascular" radioactivity rose from zero to a maximum of 10 20% of the injected dose usually attained after 1 2 days. Thereafter the curve declined in parallel with the plasma curve in those patients where the curve for the total retained activity was parallel with the

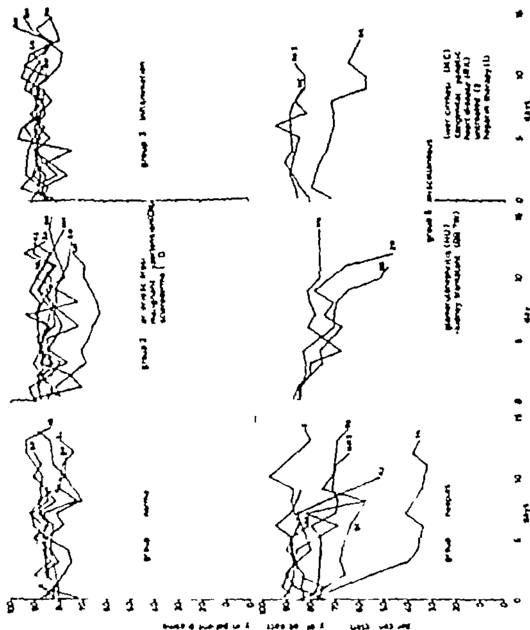


Fig. 14 Changes of the coagulability of the plasma radioactivity during the fibrinogen I-131-study for all patients. They were grouped according to their underlying disease. A significant decrease of the coagulability (unclottable protein-bound radioactivity) was observed only in one patient with malignant hypertension, in several patients with neoplastic disease, in both patients with kidney transplant rejection and in the patient with congenital cyanotic heart disease. In the latter, the coagulability of radioactivity in plasma did not decrease while on heparin therapy.

Table 7 Cumulative urinary excretion of radioactivity

	per cent of injected dose eliminated at			
	24 hours	4 days	6 days	8 days
Group 1 (n=5)	14.6 (10.7 17.3)	53.6 (49.5 56.2)	62.0 (58.0 65.9)	68.1 (65.1 74.6)
Group 2 (n=6)	16.7 (8.6 26.4)	60.5 (47.8 77.1)	74.6 (59.6 88.9)	
Group 3 (n=6)	19.4 (8.1 32.4)	64.0 (54.7 75.0)	74.5 (86.6 82.2)	84.0 (80.4 86.5) n=3
Group 4 (n=4)	17.1 (13.0 28.0)	64.8 (51.0 88.8)		
Group 5 (n=4)	13.6 (6.3 20.4)	64.0 (46.2 73.5)	76.7 (63.0 90.3)	84.8 (72.3 96.5)

plasma curve. In the other cases, the extravascular activity decreased more slowly than the plasma activity a possible interpretation being fibrin deposition.

- *The TCA-soluble radioactivity in plasma* (fig. 6-9) was below 1 2% throughout the study and its decrease was grossly parallel with the plasma curve.

- *Turnover rate*

  In tables 2 6 the fractional catabolic rate has been given as calculated from plasma and urine data. The average was 22.8 (16.2 26.1) per cent per day in group 1 (control individuals), 36.3 (35.0-42.6) in non-anticoagulated patients of group 2 (arteriosclerosis, scleroderma), 33.0 (26.9-38.2) in group 3 (inflammatory diseases) 51.0 in group 4 (neoplasia) and 62.6 in the last group (miscellaneous).

  The absolute turnover rate in mg/kg/day however was not maximal in the last two groups but rather in group 3 (inflammatory diseases), because these patients had the highest total plasma fibrinogen.

- *Pool size*

  When calculated from the intercept at t = 0 of the distribution curve (percentage intravascular of total retained activity) in the control group an average of 83.5% (70.6-95.9) of the fibrinogen was found intravascularly Using this figure, the total exchangeable fibrinogen was then 167 mg/kg (141 188). However the total fibrinogen (+ "fibrin") calculated from the relative amount of plasma and "extravascular" radioactivity at equilibrium time amounted to 225 mg/kg (203-246). For example in patient Z.L. (fig. 6) the plasma activity at equilibrium time was

60,7 ⅛ the extravascular activity ‿45%. The ratio between this total fibrinogen + "fibrin" and the intravascular fibrinogen as calculated from $\frac{Qp + Qe}{Qp}$ is 1.4. The reciprocal value corresponds to the Qp percentage of intravascular fibrinogen. The "fibrin" pool, equal to the difference between total fibrinogen + fibrin calculated at equilibrium time and the total exchangeable fibrinogen calculated from the distribution curve was 59 mg/kg (6-100) in the control group as compared to a total intravascular fibrinogen of 138 mg/kg and an extravascular fibrinogen of 79 mg/kg.

The total exchangeable fibrinogen was similar to the normal value in the non-anticoagulated patients of the arteriosclerotic group (average 165 mg/kg) but considerably higher in the patients with inflammatory processes (average ‿33 mg/kg) and in those with neoplasia (average 198 mg/kg). However the patient with a giant hemangioma both patients with kidney transplants and the patient with congenital cyanotic heart disease (under heparin therapy) had low values. A strikingly high "fibrin" pool was found in the patient F.M. with tuberculous pleuritis (140 mg/kg) and in patient M.W. with prostatic carcinoma during therapy with estrogens (360 mg/kg).

Influence of anticoagulation

Patient R.K. of group 5 who had polycythemia secondary to congenital cyanotic heart disease was subjected to two separate turnover studies, one without anticoagulation the under heparin therapy. The tra... as appear from table 6 the co... t results form table 8. While on hep... therapy plasma fibrinogen was h... and the half-life of the labelled f... gen longer than during the pre... study although the differences are ... tistically not significant. No compa... son could be made for turnover ... te and pool size in this patient because ... inadequate urine collection during th... first study period.

3 arteriosclerotic patients (table 3) were on prophylactic oral anticoagulants during the study and their Quick value was in the therapeutic range of 15 30%. The average plasma t/2 of I[131] fibrinogen was 3.8 days as compared to 3.2 days in the other 5 patients of this group. Due to the small number of cases, this difference is not significant. Their fractional catabolic rate of fibrinogen, their "fibrin" pool were not different from those in the other patients.

– *Influence of fibrinolysis inhibition*

Patient T.H., suffering from metastases of a prostatic carcinoma with the syndrome of intravascular coagulation (table 5) was studied while on therapy with ε amino caproic acid (fig. 1) which clinically controlled the bleeding tendency. This inhibitor in adequate dosage did not prevent the rapid disappearance of labeled fibrinogen (plasma t/2 of 1.5 days).

Table 8. Results of coagulation and fibrinogen I^{131}-studies in patient R.K., 30 yrs., with congenital cyanotic heart disease

	Oct. 1 1968	Oct. 4	Oct.22	Oct.22 Nov 4	Nov.25	Nov.26- Dec. 9	Dec.10	Dec.11	Dec.12	Dec.13
Quick, per cent	76	70	90	NO THERAPY	80	HEPARIN INFUSION	80	76	72	64
Recalcification time, sec.	107	100	100		141		125	165	113	
Residual prothrombin, per cent		< 4	< 4		< 5		< 4	< 4		< 4
Fibrinogen, mg per cent										
– Clauss method	185	300	225		250		230	250	230	230
– in Trasylol plasma					230			250	230	230
– Schultz method								240	300	270
– Biuret method			165	165±31	140	230±60	210			
Factor II per cent		84	36	(125-220)	84	(140-315)	88	63	94	62
Factor V per cent	62	98	34		72		96	62	43	47
Factor V in Trasylol plasma		84			64		92	58	47	45
Factor VII-complex, per cent		86	31		70		70	70	70	70
Factor VIII per cent			24		44		60	76	47	74
Factor X, per cent		56	43		58		65	64	58	74
Thrombin time, sec.	18	13	13		13	26->60	15	16	19	15
Euglobulin lysis time, hrs.					3.5		> 4	> 4	> 4	> 4
Fibrin plate, mm^2		0			0		0	0	0	0
Fibr. split products										
– in plasma		pos		Fibrinogen-I^{131} Plasma t/2 = 2.3 days*		Fibrinogen-I^{131} Plasma t/2 3.2 days*	neg	neg	neg	neg
– in serum	pos	pos					neg	neg	neg	neg
– in Trasylol-plasma		neg					neg	neg	neg	neg
– in Trasylol-serum		neg					neg	neg	neg	neg
Plasminogen, per cent		71								
Platelets, per mm^3 x 1000	121	131	131		113		131	163	153	153

* 95 % confidence limits: 2.1 2.6 days; 2.3 3.1 days

** the slope difference of the lines fitting the experimental data is statistically not significant.

– *Estrogen therapy in prostatic carcinoma*

In patient M.W (fig. 15 table 5) studied before and 12 days after onset of estrogen therapy for a prostatic carcinoma, that had not led to the clinical and laboratory signs of intravascular coagulation, an almost unchanged plasma half-life, an increased catabolic rate and a massively increased fibrin" pool was found while on therapy Adequate urine collection was secured by a vesical catheter.

b) Surface radioactivity

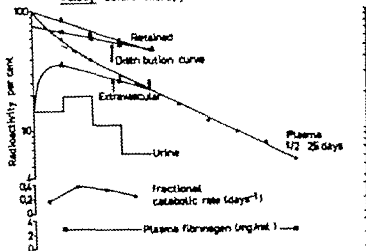
In 6 patients daily surface radioactivity measurements were done in addition to plasma and urine collection. In some continuous counting of surface radioactivity was also performed during the first hour after injection of the tracer dose. The surface data were corrected for radioactive decay and background.

– *Giant hemangioma*

The data obtained in this patient are given in tables 5 and 9

Case report: This 30 yr old man was born with a big hemangioma in the region of the right buttock and the right hip. A radiotherapy in the first years of life was not successful. At the age of 3 she had a pathological fracture of the right femur, the right lower extremity remained hypoplastic. In 1963 ischaemic complications developed in the right foot later followed by circulatory problems in the whole leg, with cyanosis and reduced skin temperature Because the toes became gangrenous in 1966, and because of further progression of ischemia, the right lower extremity was amputated by exarticulation in the coxa on May 24 1968 Dissection had to be done across the huge hemangioma, which could only partially be removed. The patient almost bled to death and required a total of 30 blood transfusions during the operation. No preoperative coagulation analysis was performed. During operation hypofibrinogenemia was found without evidence for increased fibrinolytic activity (table 9). Fibrinogen was normal for three days following the operation, due to massive transfusions. The patient then recovered, had no more bleeding tendency except in the highly fragile region of the operative field The fibrinogen level fell again and was stable at an average of 90 mg% during the fibrinogen turnover studies, when no bleeding occurred.

Surface counting during the first hour following injection of I^{131} fibrinogen showed a high activity of the order of magnitude observed in the precordial region, directly over the rest of the hemangioma. However no further and preferential accumulation of the tracer could be conclusively shown. A whole body scan, one hour following injection revealed a region of high activity corresponding to the right buttock, but no other regions with abnormal accumulation of radioactivity Daily measurements demonstrated an unequivocal accumulation of the tracer in the right buttock as compared to the left one (fig. 16). A subsequent study



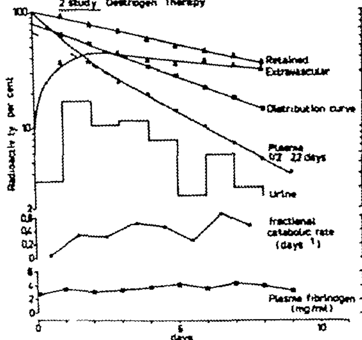


Fig. 15 Fibrinogen I^{131}-study in patient with metastatic carcinoma of the prostate, but without evidence for intravascular coagulation. The study was repeated after institution of estrogen therapy which apparently did not influence the results. Despite absence of coagulation changes indicating intravascular coagulation, this patient had a markedly shortened t/2 of plasma fibrinogen and an increased catabolic rate.

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In patient M.W (fig. 15 table 5) studied before and 12 days after onset of estrogen therapy for a prostatic carcinoma, that had not led to the clinical and laboratory signs of intravascular coagulation, an almost unchanged plasma half-life an increased catabolic rate and a massively increased "fibrin" pool was found while on therapy Adequate urine collection was secured by a vesical catheter.

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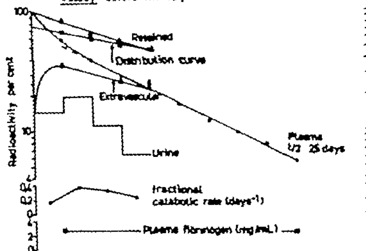
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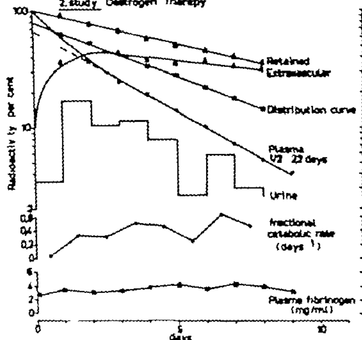


Fig. 15 Fibrinogen I^{131}-study in a patient with metastatic carcinoma of the prostate, but without evidence for intravascular coagulation. The study was repeated after institution of estrogen therapy which apparently did not influence the results. Despite absence of coagulation changes indicating intravascular coagulation, this patient had a markedly shortened t/2 of plasma fibrinogen and an increased catabolic rate.

Table 9 Blood coagulation in patient S.T f., 30 yrs., with a giant hemangioma of the right thigh

	Amputation → May 24 1968	May 25	May 27	May 29	August 12	Aug. 13	Aug. 21
Quick, per cent	46	52	61	54	60		
Recalcification time, sec.	159	102	99	95	83		
Residual prothrombin, per cent	< 4	< 4	< 4	< 4	< 4		
Fibrinogen, mg. per cent							
– Clauss method	105	210	210	155	80		
– " in Trasylol plasma					74		
– Schultz method					<120		
– Biuret method					82		
Factor II per cent	50	39	58	54	52	Fibrinogen-I^{131}-turnover Plasma t/2 = 1.6 days	
Factor V per cent	52	37	60	52	>100		
Factor V in Trasylol plasma					>100		
Factor VII-complex, per cent	68	47	82	66	62		
Factor X, per cent	30	58	80	40	–		
Thrombin time, sec.	17	15	16	17	21		
Englobulin lysis time, hrs.	> 4	> 4			> 4		
Fibrin plate, mm^2	0				0		
Fibr. split products							
– in plasma	pos	neg	neg		neg		
– in serum	pos	neg	neg		neg		
– in Trasylol-plasma							
– in Trasylol-serum							
Plasminogen, per cent					45		
Platelets, per mm^3			38 000*		217 000		

Massive hemorrhage during operation 30 transfusions during operation and 17 transf

** May be influenced by prior massive blood transfusions

using I^{131}albumin gave no accumulation of the radioactivity in the hemangiomatous tissue, although the surface scan obtained one hour after injection was identical with that after I^{131}-fibrinogen. Thus, the initially observed high activity over the hemongioma is due to the intense vascularization, whereas the further preferential accumulation of radioactivity in this tissue is only observed with fibrinogen. Unfo tunately the patient did not agree t volunteer for a further study with I^{13} fibrinogen on heparin therapy

– *Tuberculous pleuritis with fibrinou exudate*

In patient F.M. (table 4) counting on the right wall showed some

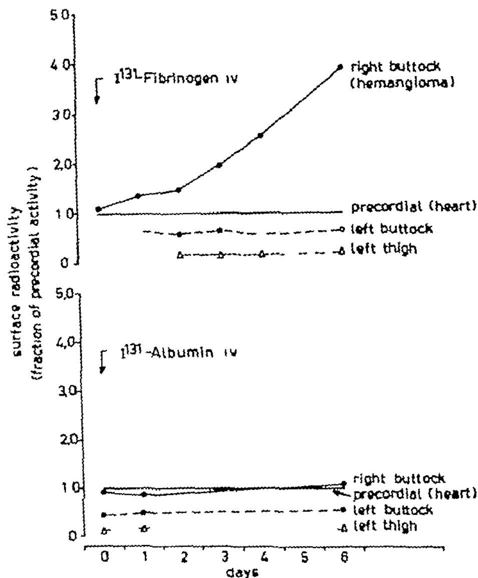


Fig. 16 Surface radioactivity following injection of I-131-fibrinogen. A preferential localization of radioactivity in the region of the hemangioma was only observed after I-131-fibrinogen and not in control experiments with I-131-albumin.

tion of the label on the side of the pleura exsudate (fig. 17) This patient with active tuberculous pleuritis also had a shortened half-life of plasma fibrinogen (t/2 = 2.5 days), an increased catabolic rate and a considerable "fibrin pool (table 4)

– *Kidney transplants*

Case reports. Patient B.B., 29 yrs, was nephrectomized in November 1967 because of chronic uremia due to pyelonephritis. In December 1967 a cadaver kidney was transplanted which functioned satisfactorily until January 4 1968 when it had to be resected because of ischemic necrosis of the transplanted ureter February 4 1968 another cadaver kidney was transplanted into the left fossa iliaca. The transplant functioned immediately following operation, with a daily urine volume of 2500 ml and excretion of 1200 mOsmol per 24 hours. The fibrinogen I^{131}-study was performed during a phase of decreasing urine volumes, increasing blood creatinine and urea (fig. 18) which were undoubtedly due to a chronic rejection of the transplant. This was confirmed by the fact that following irradiation of the transplant, and injection of twice 200 ug of Actinomycin-C at the end of the study the urine volumes promptly increased to a mean of 2500 ml.

Patient Z W., m., 43 yrs, had recurrent tonsillitis in childhood, and was observed since 1965 because of polydipsia, fatigue nausea and hypertension. From July 1967 he had a chronic peritoneal dialysis because of oliguria. January 10 1968 he was nephrectomized bilaterally the histological diagnosis being chronic glomerulonephritis. February 4 1968 after another period of peritoneal dialysis, a cadaver kidney was transplanted into the left fossa iliaca. After a short period of excellent urine excretion, the patient had a rejection phenomenon around February 10, 1968 (fig. 19) Following an increase of the prednisone dosage and 3 injections of actinomycin-C the urine volumes increased, but from February 18 through March 1 a decrease was again noted with a rise of the blood urea to 174 mg% and of creatinine to 1 7 mg%. Following local radiotherapy intravenous actinomycin-C and increase of the prednisone to 200 mg daily blood urea and creatinine returned to 97 and 1 1 mg% respectively on March 9 Thus, the fibrinogen turnover study was performed during a short phase of increasing, then during a phase of unequivocally decreasing renal function.

Fibrinogen turnover studies in both patients (table 6) revealed a surprisingly low plasma fibrinogen, a reduced total fibrinogen, a greatly reduced plasma t/2 of I^{131} fibrinogen and a massively increased fractional catabolic rate (table 6) Surface radioactivity above the transplants increased from 25 to 92 and from 28 to 90 %.

– *Glomerulonephritis*

In a third patient with renal disease H.U (table 6 fig. 11), who had acute glomerulonephritis, fibrinogen half-life

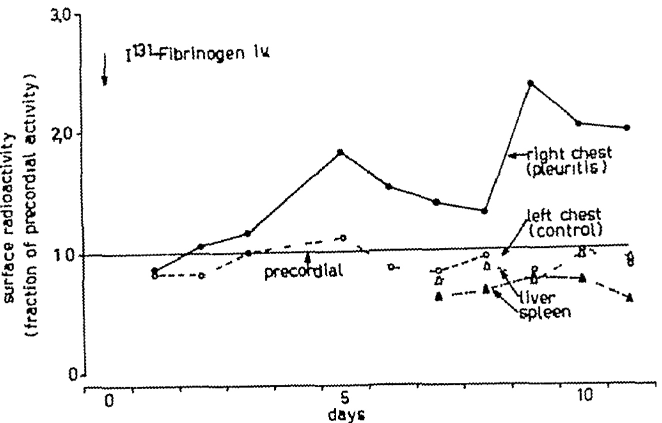


Fig. 17 Surface radioactivity following injection of I^{131}-fibrinogen in a patient with tuberculous pleuritis. Radioactivity appears to be accumulated in the region of the pleura effusion.

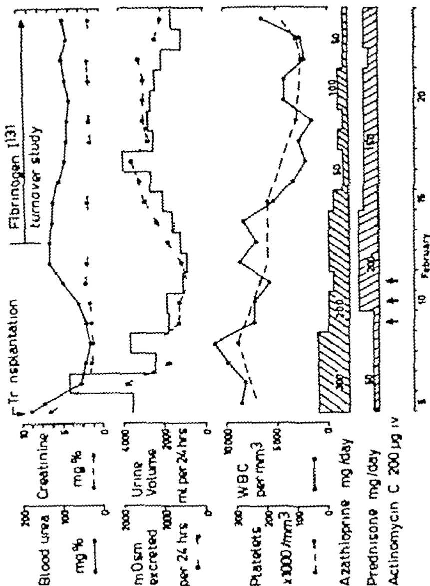


Fig. 19 Laboratory results in the second patient (Z.W.) with kidney transplant. Decreasing urinary volumes during the second two thirds of the period of the fibrinogen turnover study suggest an incipient transplant rejection.

was also shortened (9 days); the catabolic rate (59.0%/day) and the total exchangeable fibrinogen (90 mg/kg) were markedly increased. Surface counting showed a moderate accumulation of radioactivity (in per cent of the precordial radioactivity) within 9 days form 57.3 to 105.6 over the left and from 57.3 to 100.6 over the right kidney.

- *Liver cirrhosis*

In patient M.C. with liver cirrhosis, surface measurements of radioactivity over the liver did not show a [illegible] accumulation as compared with the precordial region and the [illegible] [illegible]

- [illegible]

[illegible]

[illegible]

the 10, 20 and 30 minutes samples and not as *fractions of the 10 or 15 minutes* samples (42,297). Since the loss during the first 10 minutes was 4% the actual extrapolation value in the present study was 61 7%.

An *early loss of iodine from denatured* protein results in an early accumulation of free I^{131} in the plasma and a deformation *of the curve for the radioactivity re*maining in the body (4,194,297). In our cases, plasma free I^{131} did not exceed 1.5% and there was no undue excretion of radioactivity during the early phase of the study The fraction of radioactivity ex creted during the first 24 hours in our "normal" persons was 14.6%, as compared *to 14.5% in the study of Takeda.*

It has been suggested, that degradation of the tracer fibrinogen leads to false high values for plasma volume (297). Although we have not checked the plasma volume with other methods in these patients, the average values obtained with I^{131}-fibrinogen *in those patients of groups I 3 that* had a normal hematocrit ($47 \pm 7\%$ in males, $42 \pm 5\%$ in females) were 41.6 ml/kg in 4 male subjects and 39.0 ml/kg in 10 female subjects, as compared to a normal value of 40 ml/kg obtained with I^{131}-albumin.

Our normal plasma half-lives of 3.9 (3.6-4.5) days are comparable to those of other authors (2,10,42 195,297). Since the presence of contaminating labeled proteins with a longer half-life might com*pensate for a shortening of the half-life* due to denaturation, it is important to note, that the percent non-clottable radioactivity was determined every day throughout the study and was found constant except in 8 patients where fibrinogen degradation products most probably accounted for the difference. They were *in fact demonstrated at one time in 3 of 5* tested patients.

The plasma disappearance curve was *parallel with the curve for total retained* radioactivity as would be expected with a simple mamillary system in steady state in only 13 studies. In the others, less radioactivity appeared in the urine than would have been expected from the plasma decay curve The most obvious reason for this divergence of the curves, *incomplete urine collection, is virtually ex* cluded, since the data from those 9 studies, where urine collection was not possible or where adequate collection was doubtful, were not included in this series. Moreover in the patients with divergent curves, who were found in all groups, the *average percentage of radioactivity eliminated* with the urine by day 6 was 74.6 as compared to 73 7 in the patients with par allel curves. Thus, divergence of the curves may not be attributed to incomplete urine collection. Retention of non-protein-bound iodine in the body (4) has been excluded by daily determination of the TCA-soluble radioactivity The possibility of fibrin deposition, suggested by the accumulation of radioactivity in certain organs observed in experiments with surface radioactivity measurements, will be discussed later

The concept of intravascular coagulation being an important intermediary mechanism in a variety of diseases (201,232) is fascinating not only because of its scientific interest. For the clinician, the therapeutic consequence, namely anticoagulation, would open a wide field of action in a complication where no satisfactory therapy be it even palliative is available. Once it was established, that acquired hypofibrinogenemia, combined with thrombocytopenia and a few other laboratory findings, is often associated with demonstrable fibrin microthrombi, and may sometimes be stopped by anticoagulation, the finding of microthrombi in other conditions not necessarily associated with hypofibrinogenemia, as well as elegant experimental studies, have led to the extension of the list of diseases leading to or being the consequence of intravascular coagulation. On the other hand, in experimental intravascular
phase with increased coagul
-called hypercoagulable state
ceds the actual def
that the laboratory findings
coagulable" blood might
pending or
lation. However in this
important reservations have to
regards the clinical application
cept

1 A great number of cases has published, where the credit for ization of hypofibrinogenemia for a favorable outcome was given to the therapeutic use of anticoagulants or where a "beneficial effect" of anticoagulants was used as an argument for the presence of intravascular coagulation. Since it is now well known that acute intravascular coagulation such as observed in many gynecological complications, hemolytic reactions, the generalized Shwartzman reaction etc. is usually acute and self-limited, so that the plasma fibrinogen is spontaneously normalized within 24 hours (251,286) the above argumentation is not acceptable in cases where the diagnosis of intravascular coagulation is based on an isolated coagulation profile be it as sophisticated as imaginable

present report we to
between
ascular
may still be
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of

mental animals a beneficial effect of anticoagulants in acute intravascular coagulation has only been shown with prophylactic anticoagulation and not with anticoagulation at a time when the clotting components were already consumed.

2. Hypercoagulability" usually based on a shortening of the clotting time in one or another coagulation system, elevation above normal of one or more clotting factors or an abnormal number or behavior of platelets, is too nebulous a term (232) to be used as evidence for a process of intravascular coagulation. The clotting times may be shortened for various reasons such as physiological degrees of stress (94 150,327) lack of inhibitors (95) or activation fo fibrinolysis (222) The normal activity of the clotting factors in blood is already so much in excess, *that it has been doubted whether fur*ther enhancement is of much significance (87). In fact artificial increases do not induce intravascular coagulation (232). Finally increased numbers of platelets, although probably predisposing to local thrombosis, usually do not lead to generalized intravascular coagulation and too *many* physical and chemical factors affect the behaviour of platelets (185) to make changes of platelet aggregation or adhesion valuable in the diagnosis of impending or subclinical intravascular coagulation.

In the present study we have reported only on patients with laboratory evidence for intravascular coagulation, where the coagulation abnormalities were constant during several days or weeks and secondly on patients, all of them in a steady state, where studies with I^{131}-fibrinogen were performed in order to determine whether these diagnostic procedures alone or in conjunction with the use of anticoagulants or fibrinolysis inhibitors, might be *valuable in cases with suspected intravascular coagulation.*

I DIAGNOSIS OF CHRONIC INTRA *VASCULAR COAGULATION*

*Diagnosis of severe intravascular coagula*tion even if chronic is important because the process leads to an impairment of hemostasis which, often in the absence of spontaneous bleeding, may lead to potentially dangerous hemorrhagic accidents during therapeutic or even diagnostic interventions. Thus, one of our patients, *who had not been bleeding spontaneous*ly nearly bled to death when she was amputated for a giant hemangioma.

Furthermore spontaneous or inadvertently provoked bleeding may threaten the life of patients with an otherwise favorable prognosis. Even in patients with cancer considering the prevalence of prosta*tic carcinoma, cases who had severe he*morrhagic complications may have a long survival with chemotherapy This is illustrated by one of our patients with advanced prostatic carcinoma, who had a severe chronic defibrination syndrome in 1964 and is still free of symptoms under estrogen therapy Finally the diagnosis of *intravascular coagulation may be the first* indication for the presence of unrecognized malignant disease (121,171,213)

a) Symptoms and laboratory findings

Because many symptoms often associated with acute intravascular coagulation such as hypotension, cyanosis (264) disturbances of consciousness (323), oliguria etc., are usually absent in cases with chronic intravascular coagulation the in vivo diagnosis may be much more difficult. It appears that a new steady state is reached where continued fibrin deposition must be met by a continuous removal of fibrin, thus preventing the establishment of ischemic lesions, which are so often found in cases with acute intravascular coagulation. In some of the patients therefore, no symptoms at all are found, and thrombocytopenia, or a low Quick value were the only clue to the diagnosis in several of our cases. The coagulation analysis than revealed hypofibrinogenemia and a variable and inconsistent reduction of factors II V and VIII and sometimes also of the vitamin K dependent clotting factors VII IX and X. Direct evidence for fibrinolysis was found in only a few patients, indirect evidence (fibrin degradation products, reduction of plasminogen) in many of them. These features have been discussed by others (57,156,174,232,252,256,292,313)

The exclusion of other causes of severe hypofibrinogenemia is usually not difficult (256). *Congenital afibrinogenemia is not associated with deficiencies of other clotting factors or thrombocytopenia.* Of the two other major causes of acquired *hypofibrinogenemia, decreased production* by the liver may be rapidly ruled out since only the most severe cases of hepatic necrosis, such as fulminant hepatitis in the comatous stage demonstrate a production defect sufficient to cause hypofibrinogenemia (285) and this at a time when all the other clotting factors have already reached minimal levels which are not observed in *intravascular coagulation.* A primary chronic systemic fibrinogenolysis of a degree leading to *hypofibrinogenemia* has been described in the terminal stage of liver cirrhosis, which can be diagnosed clinically The proteolytic state of patients with metastatic prostatic cracinoma, which has long been the most important example of a primary fibrinolytic state (295,296) probably is secondary to intravascular coagulation in most cases, as will be discussed below

The histological diagnosis rests on the demonstration of *fibrin microthrombi* using various techniques (200) Again, this is reliable only when positive The recent work of Bleyl and coworkers (36) suggests that the currently used techniques for fixation may not be entirely adequate In many patients, nothing is found either in biopsy specimens or at autopsy Indeed, from a theoretical standpoint occlusion of the microvasculature by fibrin thrombi would lead to a catastrophe such as observed in acute intravascular coagulation, whereas continuous fibrin formation and deposition over a long period of time and of a degree leading to hypofibrinogenemia is not thinkable without a very potent mechanism for fibrin disposal. The absence of histological evidence for generalized fibrin deposition in these states is therefore not surprising. The disposal of fibrin appears to be undertaken by

1) the reticuloendothelial system, which has a very high capacity for the clearance of clotting intermediates (?77) and of fibrin (82,176,177) and possibly

2) the enzymatic proteolysis of deposited fibrin by a localized or systemic activation of fibrinolysis (106)

Whereas the first mechanism can only be demonstrated using highly elaborate techniques, *the second gives rise to circulating* fibrin degradation products easily detectable in patient blood. Using a not very sensitive assay we could demonstrate circulating fibrin degradation products in many cases, even where simultaneous tests *for systemic fibrinolysis were negative*

Others have made similar experiences (156,204) and a local activation of plasminogen to plasmin was postulated to occur at the site of fibrin deposition on the endothelium (106) In fact, endothelial *cells have been shown to have a high fibrinolytic* potential (302,321). Therefore when this test is not made too sensitive e.g. does not give false positive results in normal persons under physiological conditions (74) a positive test offers indirect *evidence for intravascular coagulation*, especially in patients where no systemic fibrinolysis is found. In the latter cases, most often patients with carcinoma of the prostate the possibility of a primary fibrinolysis instead of fibrinolysis secondary to intravascular coagulation has always to be considered. The laboratory differential diagnosis (86) is based on the fact, that plasmin in vitro consumes fibrinogen, factors V and VIII but neither prothrombin and the other vitamin K dependent clotting factors (159) nor the platelets in vivo (86), whereas clotting in vitro leads to consumption (conversion) of fibrinogen, factors V and VIII but also of prothrombin and platelets. Although in vitro factors VII IX and X are not consumed during coagulation, in vivo an unexplained drop has been found in cases with acute intravascular coagulation and also in animal experiments (243) A similar observation has been made in our cases with chronic intravascular coagulation.

Very recently a rapid screening test for the detection of intravascular coagulation has been proposed which is apparently negative in cases of pure fibrinolysis (54) It is based on the observations, *that fibrin monomers may circulate in a* soluble form (268) that soluble fibrin may appear in the circulation in cases of intravascular coagulation (124) and that these fibrin monomers can be precipitated (gelated) by addition of ethanol to plasma *(125) According to our own preliminary* results, this test is not very sensitive but proves to be useful in severe cases of intravascular coagulation. Others have attempted to develop a test using protamin (81 182) which precipitates complexes of fibrin monomers with fibrinogen.

Finally the presence of fibrin monomers leads to a cryoprecipitability of the plasma (cryofibrinogen) (268) Cryofibrinogen has been found in patients with chronic intravascular coagulation (45 171,213,330) The fact that this cryoprecipitate diminished during heparin therapy and reappeared after its cessation (170,171,213) is an argument for intravascular coagulation being the cause of its appearance. In all 3 patients of our own series, where a cryoprotein test was done, cryofibrinogen could be demonstrated. This simple procedure may therefore give additional indirect evidence for intravascular coagulation. Since platelets may be reduced by bone marrow involvement es-

pecially if chronic intravascular coagulation is due to neoplasia, as in most of our cases, the question whether fibrinolysis is primary or secondary often remains controversial in a given patient. In such cases, and provided that a steady state is present, the use of fibrinolysis inhibitors or of anticoagulants may be of diagnostic usefulness.

b) Diagnostic use of anticoagulants and fibrinolysis inhibitors

Heparin has first been used as a therapeutic agent in intravascular coagulation by Little in 1959 (184) Later "heparin treatment of bleeding" has been advocated by several authors (312,317) but only in 1964 Merskey and coworkers (204) clearly used anticoagulants as a diagnostic tool in a patient with cancer of the colon and hypofibrinogenemia. Heparin normalized the fibrinogen level on several occasions. In 1967 we have shown, that heparin suppressed systemic fibrinolysis on four separate occasions in a patient with prostatic carcinoma, thus, suggesting that fibrinolysis, though quite intense may be secondary to intravascular coagulation (287). Oral anticoagulants have also been used, at first unsuccessfully (124,273,304) until it became clear that an effect on the process of intravascular coagulation can only be expected with Quick values below those recommended for the conventional use of these drugs (38,40,328). It has now been established in a number of patients, that both types of anticoagulants may be effective in the same patient (16,40) To be used as a valid argument for the presence of intravascular coagulation in a given patient, three conditions should however be fulfilled.

1) The patient has to be in a steady state e.g. has to have chronic and not acute hypofibrinogenemia,
2) systemic fibrinolysis should be immediately suppressed and
3) fibrinogen/fibrin breakdown products with their plasma half-life of only 9 12 hours should disappear within a day or two after initiation of therapy Presuming a normal synthesis of fibrinogen by the liver the fibrinogen level may take somewhat longer to normalize.

Intravascular coagulation being not affected per se by fibrinolysis inhibitors, the suppression of the reactive fibrinolysis should theoretically not affect the rapid disappearance of fibrinogen from plasma in these cases. We have been able to demonstrate this in three of the present patients with hypofibrinogenemia, in two of them using massive infusions of unlabelled fibrinogen, in another using a tracer dose of I^{131} labelled fibrinogen. However it has been reported (12,283) that in patients with prostatic carcinoma and hyper fibrinolysis, ε amino caproic acid therapy may lead to a normalization of the fibrinogen level. Since heparin therapy suppresses the fibrinolysis (287) there is little doubt, that intravascular coagulation is the primary process. The normalization of the fibrinogen level by ε amino caproic acid can then only be explained by suppression not only of the reactive fibrinolysis, but also of a systemic fibrinogenolysis. In fact, the intensity of the systemic fibrinolytic process in some cases of prostatic carcinoma is such that fibrinogeno-

lysis is very likely. Thus, normalization of the fibrinogen level during therapy with a fibrinolysis inhibitor does not exclude the presence of a process of intravascular coagulation. In this situation, only survival studies with labelled fibrinogen can demonstrate the continued consumption of fibrinogen, as observed in one of the present patients. It may be concluded, that fibrinolysis inhibitors may be of diagnostic interest in situations with suspected intravascular coagulation. However in this connection, the possible hazard of fibrinolysis inhibitors in a situation where fibrinolysis is only reactive and teleological ly has the function of removing pathological fibrin deposits, has to be mentioned (30). On the other hand, reports of thrombotic complications have been comparatively rare (64 155,217) and are at variance with the reports of obvious clinical benefit with control of the bleeding tendency in patients with strong fibrinolysis, notably cases with prostatic carcinoma (12,56). Based on the above explanation of the normalization of the fibrinogen level despite continuing intravascular coagulation, one may suppose that suppression of fibrinogenolysis with or without normalization of the fibrinogen level could possibly suffice for the control of bleeding in some patients.

For the above reasons, fibrinolysis inhibitors should not be used except in cases where systemic fibrinolysis is prominent

2. STUDIES OF FIBRINOGEN METABOLISM DISTRIBUTION AND LOCALIZATION USING I^{131}-FIBRINOGEN

a) Turnover studies

Fibrinogen production

In 1963 we have demonstrated in vitro fibrinogen formation by liver slices (284) and subsequently Barnhart and coworkers (22) have been able to localize fibrinogen in liver cells using an immunofluorescence technique thus confirming earlier evidence for fibrinogen production by the liver which was based on the rapid drop of circulating fibrinogen following hepatectomy or toxic liver injury (84,223). The in vitro incubation studies with liver slices indicated a production of 1.65 gr daily which agrees well with results of perfusion studies with rat livers (207) and turnover studies (4). The perfusion experiments of Miller (206) also suggested, that the production rate is a function of the fibrinogen level of the blood reaching the liver and that production could be increased by a factor of 6 by lowering the *fibrinogen of* the perfusate.

Fibrinogen catabolism

Like the other clotting factors, fibrinogen has a much shorter half-life than most other plasma proteins. Survival studies using fibrinogen labelled with I^{131} or I^{125} (2 10 19 42 65 131 135 152 194 195 236 245 297 300), S^{35} (187,316) C^{14} (235,236) and Se^{75} (115) in normal subjects have given essentially similar re

sults as studies using infusions of unlabelled fibrinogen in congenitally afibrinogenemic subjects (123 127,325) The disappearance curves always demonstrate a double exponential pattern the first component representing equilibration with the extravascular space the second component from day 1 2 onwards representing metabolic degradation (4). The results obtained in our control group (plasma t/2 of 3 6-4.5 days) *perfectly agree* with those of previous studies. Earlier observed longer halflives (65 131 187) have probably been due to reutilization of the label (187) or partial denaturation of the in vitro labelled fibrinogen (297). When certain precautions are made (4 42,297) turnover studies using fibrinogen labeled in vitro with iodine isotopes yield reliable results. Takeda (297) has inferred that autologous fibrinogen should give more accurate results than fibrinogen isolated from a plasma pool. Whereas this may be acceptable for normal individuals, labeling of the patients own mixture of fibrinogen and partly degraded fibrinogen and fibrin in patients with chronic intravascular coagulation or fibrinolytic states may yield inadequate results. Our own data in patients with kidney transplants, where the decrease of coagulability of the plasma radioactivity during the study without concomitant increase of the TCA-soluble radioactivity indicated the continuous dilution of clottable fibrinogen with unclottable derivatives (117), suggest that at least in one study of patients with nephrosis (298), the use of autologous fibrinogen may be questionable This difficulty may be partially circumvented by isolating the fibrin of each plasma sample (42). In the present study we have used homologous fibrinogen and determined the clottability throughout the study

The exact mechanism of the physiological catabolism of fibrinogen is not known. The intriguing question of whether fibrin is continuously formed, constituting a physiological lining of the entire vascular endothelium (253) and that excess fibrin is continuously removed by *either fibrinolysis* (14) *or the reticulo-endothelial* system (176) is still not resolved (144) However since anticoagulation (178) a hemophilic state (2,42,245,299) and also fibrinolysis inhibition with ε -amino caproic acid (114) did not measurably influence the turnover of fibrinogen in normal animal or man, other mechanisms of catabolism appear to exist, and it seems unlikely that intravascular coagulation is merely an exaggeration of a physiological process. Recently Sherman and coworkers (271) comparing the turnover of intact and partly degraded fibrinogen, have postulated that the major portion (roughly 75%) of fibrinogen is catabolized by a pathway other than the plasminogen-plasmin proteolytic enzyme system. It must also be remembered that *the catabolic rate of fibrinogen might be* governed by the rate of synthesis rather than vice versa (298).

As expected our patients with laboratory evidence for intravascular coagulation (1 patient with prostatic carcinoma, 1 with promyelocytic leukemia and 1 with a giant hemangioma) had a markedly shortened plasma t/2 of labelled fibrinogen. In the same cases, the t/2 of clottable fibrinogen (fibrin) was even shorter a finding which was due to a decrease of the

coagulability of the protein-bound plasma radioactivity One of these cases had a shortened half-life despite continuous therapy with ε -amino caproic acid In addition, almost identical findings, namely a markedly shortened t/2 of labelled fibrinogen and an even shorter half-life of labelled isolated fibrin, have been found in 5 patients without conventional laboratory evidence for intravascular coagulation namely in one patient with metastatic carcinoma of the ovary one patient with metastatic carcinoma of prostate in both patients with chronic rejection of a kidney homograft and in a patient with congenital cyanotic heart disease In the latter case, the repetition of the study under heparin therapy showed a higher average plasma fibrinogen and a t/2 of 3.2 days instead of 2.3 days before heparin. In these cases, the postulate of a subclinical process of intravascular coagulation may be justified

The shortening of the half-life of I^{131} fibrinogen below 3 days in two other patients with metastatic carcinoma of unknown origin, in one patient with arteriosclerosis, 3 patients with inflammatory processes and 1 patient with glomerulonephritis was not associated with an obvious decrease of the coagulability of plasma radioactivity In these last cases, interpretation has to be cautious and the results certainly may not be ascribed to a process of intravascular coagulation unless other arguments such as normalization by anticoagulants etc can be put forward.

McFarlane et al. (195) have found that in various diseases the fractional turnover rate the half-lives and the ratio of intravascular to extravascular fibrinogen did not vary significantly and that increases in the absolute turnover of up to 100% as in pulmonary tuberculosis were due to changes in plasma volume and notably in fibrinogen concentrations. Fibrinogen catabolism would then be a first order kinetic process (4,246) e.g. as the concentration increases the per cent catabolized remains constant but the total amount catabolized increases According to our results, an increase of the absolute turnover rate above 40 mg/kg (control group 21.9 38 7 mg/kg) was associated with a grossly normal fractional catabolic rate of less than 30% per day (control group 16 ‑26% per day) in only 3 patients, 2 with inflammatory processes (B W with bronchopneumonia and B.J with Landry paralysis) and 1 with prostatic carcinoma (M W). The high absolute turnover rate was due to both a high plasma fibrinogen and a high plasma volume in the first and the third patient, to a high plasma volume alone in the second case. In all the other cases however the increase of the absolute turnover rate was due to either an increased catabolic rate (notably in the cases with chronic intravascular coagulation) or to a combination of increased catabolic rate and increased total plasma fibrinogen. Fibrinogen levels below 250 mg% were only observed in patients with considerable to excessive increase of the fractional catabolic rate. Thus, it appears from our results, that a low plasma fibrinogen level is necessarily associated with a high catabolic rate and that a high plasma fibrinogen level is not due to a decreased catabolic rate but may be associated with a normal or increased catabolic rate. The only qualitative difference

travascular coagulation. The syndrome may be observed in the newborn (140,312) as well as in the adult (119) and has also been demonstrated in mice following transplantation of hemangioendotheliomas (146). In the present report we contribute a strong argument for intravascular coagulation, namely the massive preferential accumulation of radioactivity in the region of the hemangioma after injection of I^{131} fibrinogen. The hypofibrinogenemia and the rapid disappearance of labelled fibrinogen from the circulation were also confirmed. Recognition of this syndrome is important because

1) hemangiomas may be intracerebral (260) or intraabdominal (25) thus not early diagnosed, and still cause a severe hemorrhagic diathesis. In such cases, coagulation analysis will raise the suspicion. As observed in our patient, the highly vascularized tissue can easily be demonstrated by surface scanning after administration of any blood tracer. The advantage of labelled fibrinogen obviously lies in our demonstration of preferential accumulation of fibrinogen in contrast to radioactive albumin. Using these methods, occult hemangiomas should be easily and accurately diagnosed. In fact, using our fibrinogen preparation, Gugler (128) has been able to demonstrate an intraabdominal hemangioma in a girl with a chronic defibrination syndrome

2) *In a young patient in whom malignant* neoplasms, notably leukemia, has been excluded, the findings of chronic intravascular coagulation should lead to a search for an occult hemangioma as the most probable cause of the coagulation abnormality

Other non-neoplastic conditions where a more protracted defibrination has been documented include purpura fulminans the dead fetus syndrome and possibly aortic aneuryems.

– *Purpura fulminans*

The literature on this subject has been reviewed by Hjort (143). This rare complication of a variety of diseases (24 143 169) is one of the examples of chronic intravascular coagulation where anticoagulant treatment has first been used successfully (184) and where heparin (8 13 143,304) has shown its most striking beneficial effect. This is illustrated by a case observed in 1964 in whom the clotting parameters were normalized on several *instances by heparin and where discon*tinuation of the therapy immediately led to a severe recurrence of the skin symptoms and laboratory signs (304). However a case has also been published in whom heparinization was apparently unsuccessful (228)

– *Chronic defibrination in obstetrics*

Defibrination in obstetrics, such as amniotic fluid embolism premature separation of the placenta or septic abortion (314) is usually acute and self limited. However retention of a *dead*

fetus can lead to a more chronic acquired hypofibrinogenemia with all the characteristics of intravascular coagulation and a hemorrhagic state not limited to the genital tract (109,244). Most patients deliver spontaneously within three weeks of fetal death and have no bleeding problems. After more than one month retention however the incidence of hypofibrinogenemia is 1/4 1/3 of patients (147 154) and weekly determinations of fibrinogen are indicated, starting form the second week (147) Necrotic material of the degenerated placenta or of the macerated fetus may play a role in the development of hypofibrinogenemia and in fact clot promoting substances have been reported to be demonstrable in blood of such women (?74). After removal of the dead fetus, fibrinogen rapidly normalizes. Serial coagulation studies before during and after heparin therapy of such a patient confirmed the notion that the primary defect is intravascular coagulation (74) Moreover fibrinolysis is usually not a prominent finding (51,234,314). The patients may be either anticoagulated until spontaneous delivery or the fetus may be removed as soon as the fibrinogen level, which usually drops by 50 mg% per week (314), is below 200 mg%. An isolated case with *hydatiform mole* (314) has also been published, who had a bleeding diathesis during two months and who showed hypofibrinogenemia and thrombocytopenia which disappeared after operation. Finally Hnat (145) has presented convincing evidence for chronic intravascular coagulation developing during pregnancy in a patient with a degenerating myoma of the uterus.

– *Aortic aneurysm*

One of our patients with chronic defibrination syndrome (H.P.) observed 1967 has been presented in more detail. She had an aortic aneurysm, hypofibrinogenemia with thrombocytopenia, but on repeated occasions the direct assays for increased systemic fibrinolysis (euglobulin lysis time fibrin plate) as well as the search for fibrinogen/fibrin degradation products gave negative results. During life the clotting defect was attributed to intravascular coagulation associated with undiagnosed metastases of a breast cancer removed 9 years previously To our surprise autopsy revealed no metastases a liver cirrhosis and the aortic aneurysm. The hepatic cirrhosis had not led to clinical symptoms nor abnormal levels of bile pigments in urine or serum. The repeatedly normal values of factors VII and X exclude an impairment of liver function of a degree leading to hypofibrinogenemia by way of decreased synthesis. Since increased fibrinolysis occasionally observed in liver cirrhosis, has also been excluded her coagulation abnormalities have to be attributed to intravascular coagulation and the question arises whether the giant aortic aneurysm played a role in this case. In fact, a patient with aortic aneurysm has been reported (103) in whom a thrombocytopenia of 40 000/mm3 prolonged bleeding-

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By

SIGURD NITTERHAUGE, M. D

Oslo 1971

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CHAPTER I

INTRODUCTION

Myocardial infarction seems to occur with increasing frequency especially among middle-aged men. For the survivors, limitation in physical capacity may have conspicuous social and economic consequences.

Usually the post infarction patient is well compensated at rest. Studies of the ability to perform physical work would therefore be of value, yielding objective information with regard to the degree of functional impairment.

Probably the most sensitive and most physiological way of achieving a correct expression for the exercise tolerance is to study the reactions to the increased demands for O_2 uptake, CO_2 output, and pulmonary ventilation. Two different principles have frequently been used measurements of the gaseous exchange *during* an exercise performance of relatively long duration (*steady state*) and measurements of the gaseous exchange *after* exercise, usually of short duration, without achievement of a steady state.

At the University Institute for Respiratory Physiology Ullevål Hospital in Oslo, a test procedure permitting continuous recording of the O_2 uptake, CO_2 output, and pulmonary ventilation for one-minute periods before, during, and after a standardized exercise of short duration was introduced by Erikson et al. [36] and Erikson [28] and has since been used in clinical routine for several years. Attention has been paid to the recovery time for the CO_2 output, the increase in ventilation and the simultaneous increase in O_2 uptake following an exercise performance of 1 minute's duration. These parameters have been used as quantitative measurements of the total cardio-pulmonary function in healthy individuals [3, 31, 32] and in patients with various cardio-pulmonary diseases [2, 10, 28, 29 30, 33, 34, 71, 72, 73, 77]

The purpose of this study is to report on the results of these tests in men who have survived their first myocardial infarction.

SURVEY OF LITERATURE

CO_2 recovery time

The physiological processes of the respiratory gaseous exchange in man at the transition from work to rest are described in the basic works by Krogh & Lindhard [53] Hill et al. [50] Simonson [79] Liebenow [55] Hebestreit [47] Margaria et al. [59] Berg [9] and more recently confirmed by Erikson [31, 32] and Andersen [3] According to these investigators, the restitution or recovery process of the O_2 uptake and CO_2 output after a period of muscular exercise follows an exponential-like curve. After light to moderate exercise (with O_2 uptake below 2.5 lit./minute in experiments performed by Margaria et al. [59]) with little or no increase in the blood lactate, the recovery was relatively rapid, and was completed within a few minutes. After more strenuous or exhausting exercise with marked anaerobic energy release and increased lactic acid concentrations in the blood the recovery was prolonged up to hours. The duration of the recovery process, i.e. the time elapsing between the end of the exercise and the achievement of resting values, is assumed by these authors to depend on the cardio-pulmonary function. The exchange of CO_2 and O_2 between the blood and tissue is known to take place very rapidly and is not considered to represent any limiting factor [62, 74]

The duration of the recovery processes for the gaseous exchange after work increases with advancing age. In any age group, the more physically fit and better trained subjects tend to have the shortest

recovery time [3, 9 31] In experiments with work load 500 kgm in 1 minute, Andersen [3] found that the CO_2 recovery time was on an average 2 minutes shorter in physically active men than in sedentary men.

In patients with organic heart disease (mainly of valvular origin) a delay in the recovery time for the O_2 uptake has been reported by Eppinger et al. [26] Herbst [49] Banai & Groscurth [8] Nylin [68] and Katz et al. [52] In some of these experiments, the O_2 uptake due to the exercise was abnormally large (low net work efficiency) In other experiments, however the work efficiency was normal, and the increased O_2 uptake during the recovery period was assumed to compensate for a diminished O_2 uptake during the exercise period, most probably due to a lag in the circulatory response to the exercise [45, 61] The disturbances of the respiratory gaseous exchange in cardiac patients were even more pronounced when applied to the CO_2 output than to the O_2 uptake [16] The importance of cardiac disorders for the CO_2 recovery time was also demonstrated by Erikson [29] in a patient with mitral stenosis and heart failure. Prior to valvulotomy the CO_2 recovery time was 10 minutes, and was reduced to 6 minutes after the operation (work load 500 kgm in 1 minute) Refsum [71, 73] reported that a small group of patients with left heart disease in addition to pulmonary silicosis in general showed a longer CO_2 recovery time than the remaining patients.

Pulmonary ventilation and O_2 uptake

In healthy individuals performing light to moderate exercise, a rectilinear relationship exists between pulmonary ventilation and the O_2 uptake. With more strenuous or exhausting exercise, requiring more than 50—70 % of the maximum O_2 uptake and leading to accumulation of anaerobic products, the relationship between the two parameters is curvilinear in such a way that the ventilation is out of proportion to the O_2 uptake [6, 18, 22] In these studies, values for the total ventilation and the total O_2 uptake were used.

At our Institute, the ventilatory response and the O_2 uptake in connection with exercise are calculated as the differences between the total ventilation and the total O_2 uptake, respectively recorded during the exercise and the recovery period, and the resting values for the same parameters for an equal period of time. The determination of the increase in ventilation in connection with exercise of short duration is probably a more sensitive indicator of abnormal ventilation than determination of total ventilation during less severe steady state exercise. Using this method, Andersen [3] found that in healthy individuals a linear relationship between the increase in ventilation and the increase in O_2 uptake existed up to a work load of about 900 kgm. In experiments with increase in lactic acid concentration, the linearity between ventilation and O_2 uptake was broken.

The ventilatory response to exercise is known to increase slightly with advancing age [3, 43, 67] Experimental data have also shown that in otherwise healthy subjects of comparable age, the ventilatory response to exercise in physically active subjects was on an average lower than in sedentary men. With the same method as used in this study Andersen [3] found that after 500 kgm for 1 minute the increase in ventilation in elderly physically active men was on an average 11 lit. lower than in sedentary men of the same age.

In patients with cardiac diseases, an abnormally large ventilatory response to the O_2 cost of work of moderate intensity and of short duration was found by Peabody & Sturgis [69] Herbst [49] Simonson & Gollwitzer Meier [80] and Harrison et al. [46] The same was found in steady state exercise by Campbell & Sale [15] Zaeper et al. [88] Cotes [21] Donald et al. [25] and Gazetopoulos et al. [42] The striking exercise hyperventilation in cardiac patients seemed to be related to the subjective feeling of breathlessness from which these patients often suffer and was regarded as an early symptom of cardiac insufficiency even if the O_2 uptake was normal. In the most disabled patients, an abnormal resting ventilation was found by McMichael [57] and Boyer & Bally [13]

With the same exercise test as used in the present study Erikson [29] demon

strated the importance of the central circulatory system for the ventilatory increase in a patient with mitral stenosis and heart failure. Following a work load of 500 kgm for 1 minute, this patient, prior to valvulotomy had a ventilatory increase of 135 lit. which was reduced to 39 lit. after the operation. Using principally the same procedure, Fodstad [38] found that after performing 500 kgm in 1 minute, patients with varying degrees of cardiac failure had significantly higher values for ventilatory increase than healthy control subjects, but with an overlap between the two groups. When the patients were classified according to the New York Heart Association system [66] it was apparent that with progressing cardiac disability increasing values for ventilatory response to exercise were found. Refsum [73] studying patients with silicosis, observed that in subjects who suffered from definite left heart disease in addition to silicosis, average increase in ventilation was significantly higher than in the rest of the material.

The connection between the ventilatory response to the present exercise test and the haemodynamic observations obtained at rest and during moderate exercise at steady state during right heart catheterization was discussed by Eriksson & Müller [34] According to these authors, the abnormally high increase in ventilation found in a small group of patients with left heart disease (mitral valvular disease, coronary heart disease, congenital heart disease) was usually associated with an increased pressure in the left atrium and/or low cardiac output during exercise, indicating transient or permanent left heart failure.

To summarize, previous experiments at our Institute have shown that the CO_2 recovery time after exercise of 1 minute's duration can be used as a reliable indicator of the cardio-pulmonary function. The increase in ventilation in connection with the exercise test seems to be a useful indicator of circulatory insufficiency per se. In addition, the simultaneously recorded increase in O_2 uptake during the work and the recovery phase gives information on the net work efficiency Several reports have been published concerning observations in normal subjects as well as in patients with cardiac and pulmonary diseases. However the method has previously been applied only occasionally to patients with myocardial infarction.

Very scanty experimental data are available dealing with the relationship between results from the actual exercise test and haemodynamic findings.

The data from the present exercise test have only to a small extent been related to other forms of functional classification, such as clinical gradings and working ability The present study is also an attempt to provide more information in this field.

PURPOSE AND PLAN OF THE INVESTIGATION

The study deals with men below 65 years of age who had been engaged in normal working activities until the time of their first myocardial infarction. The purpose of the present investigation was to elucidate the following problems, of which present knowledge is very small or incomplete

1. To what degree is the pulmonary gaseous exchange after exercise of moderate intensity impaired a short time after myocardial infarction
2. To what extent does it change during an observation period covering the first two years after the myocardial infarction?
3. What are the relations between the data obtained from studies of the pulmonary gaseous exchange after exercise and haemodynamic findings, clinical classification, working ability and various complicating clinical conditions, and what is the effect of medical therapy?

The first examination took place 2 months after the manifestation of the myocardial infarction. It was assumed that the acute episode started when the patients developed the symptoms here used as diagnostic criteria (see page 18) Successive examinations of the same patients were carried out at 1-month intervals during the first part of the study (2 — 6 months) Afterwards, the patients were examined at intervals of 3 months.

Since the first examination took place

a relatively short time after the infarction, and since it was considered of special interest to study the mode of reaction to a work intensity which corresponded to most of the daily activities, a work load of 250 kgm of 1 minute's duration was chosen as the standard exercise. In addition, experiments using a work load of 500 kgm of 1 minute's duration were carried out 12 and 24 months after the infarction.

The material of the study consisted of the following series

a) A control series of 31 men below 65 years of age, without known cardiac or pulmonary diseases.

b) A patient series of 95 men below 65 years of age, who had been engaged in normal working activities, and who had survived their first myocardial infarction, but had suffered from no other cardiac or pulmonary diseases.

The results of the 250 kgm experiments in the patients were compared with data from similar testing of the control series. For the results of the 500 kgm experiments, data given by Andersen [3] in his examinations of healthy men of comparable age were used as reference values.

The patients included in the present investigation also participated in a study of the haemodynamics in patients with coronary artery disease conducted by Ottar Müller M.D. The haemodynamic studies were done with an ordinary right heart catheterization technique. The examination took place 3 months after the myocardial infarction.

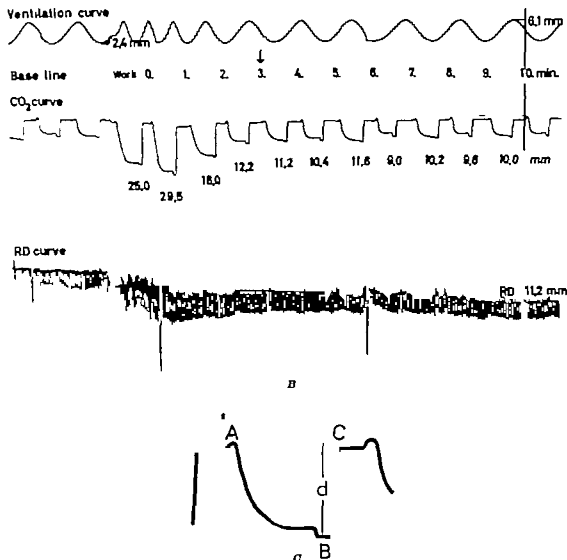


Fig. 2. Section of spirometer recording. Resting pre-exercise period (Fig. 2 A, see page 14) and working minute with recovery period with arrow marking the time when the CO_2-output has fallen to resting values (Fig. 2 B). On both sections, the top tracing (sinusoidal) shows pulmonary ventilation recorded continously by a device that summarises the inspiratory excursions, the base line below marks the time axis divided in 1 minute periods. The CO_2 curve records the volume of CO_2 eliminated in 1 minute periods. The bottom curve is the respiratory difference (RD) curve which gives the difference between the O_2 uptake and CO_2 output. The RD curve shows the respiratory frequency with inspiration downwards. Fig. 2 C shows the CO output curve (for details see text)

number of minutes from the end of the exercise to the time when the CO_2 output had fallen to resting values.

The *increase in ventilation* during the exercise performance was expressed as the difference between the total ventilation during the working minute and the recovery period minus the resting values, calculated as the average of the pre- and post-exercise level, for an equally long period. The *increase in O_2 uptake* was calculated in a similar way.

Fig. 2 is an example of the spirometer tracings.

The ventilation was recorded as a sinusoid curve. Each complete cycle was equal to

12.0 lit. Part of a cycle was determined by measuring the vertical distance from the top of the curve to the bottom.

In Fig. 2C showing the CO_2 output, A to B represents the absorption curve, whilst B to C is produced by filling the recording spirometer with oxygen. The small displacements observed at A and B are sidepressure effects when turning the blowers on and off. The CO_2 output is determined by measuring the drop of the absorption curve (d in the Figure) Each mm corresponds to 20 ml.

With regard to the respiratory difference, each mm corresponds to 50 ml.

In the example given in Fig. 2, the following results are obtained from the resting pre-exercise period (10 minutes)

Ventilation The two measured parts of the cycle 1.3 mm and 0.5 mm correspond to 1.25 lit. and 0.75 lit. respectively The total ventilation is then
(12.00 × 7 — 6.00 — 1.25 — 0 75) lit = 68.00 lit.

Total CO The sum of the CO output is (109.2 × 20) ml = 2,184 ml.

The respiratory difference (5 7 × 50) ml = 285 ml.

The total O uptake 2,184 ml + 285 ml = 2,469 ml.

The gaseous exchange and ventilation during a one-minute period are obtained by simple division.

The calculations of the data from the work and recovery period and the post-exercise period are carried out in a similar way

Accuracy

A hard graphite pencil was used for all drawings, and the measurements were performed with the help of a magnifying glass. This procedure together with the low inertia of the spirometers in the recording units and the high degree of thermostability of the whole system, made it possible to record the CO_2 output for periods of one minute with an approximate accuracy of ± 5 ml. As the O_2 uptake was calculated from the CO_2 output and the slope of the R.D.line the accuracy of O_2 determinations depended on a constant expiratory level. Pulmonary ventilation readings were made for a period of at least 10 minutes' duration, with an approximate accuracy for periods of 1 minute within ± 50 ml

Correction of gas volumes

With the spirometers, all volumes were recorded at ATPS. Volumes for the increase in O_2 uptake were expressed at STPD subtracting 10% from the recorded value. The increase in ventilation was expressed at BTPS by adding 10 to the recorded value.

Determination of the bellows function of the lungs

Determination of the bellows function of the lungs included measurements of the vital capacity (VC) and forced expiratory volume in 1 second ($FEV_{1.0}$) The examinations were performed with a spirometer with low resistance and low inertia. The measurements were undertaken with the patient in a sitting position. VC was measured as the largest volume expired from the lungs following a maximal inspiration [51] VC was recorded at ATPS and expressed at BTPS by adding 10% to the recorded value. VC as a percentage of predicted normal value was calculated from the formulae of Storstein & Voll [83] $FEV_{1.0}$ was measured as the volume of gas expelled during the first second of an expiration, and expressed as a percentage of VC [35] The highest value of two or more determinations was used as the actual value

Normal values given by Storstein & Voll [83] have been used as reference values.

Haemodynamic investigation

Right heart catheterization was performed as described by Müller & Rørvik [65] The patients were investigated in a supine position at rest and during the exercise test, which was performed on a cycle ergometer A work load of about 150 kgm/minute was used, and the exercise period lasted for 3—5 minutes. Data from examinations of healthy men of comparable age published by Müller [64] have been used as reference values for the haemodynamic findings.

Clinical classification

The functional capacity limited by angina pectoris at the time when the study started was established in personal interviews. The

CHAPTER III

MATERIAL

Control series

The control series used for the 250 kgm experiments consisted of 31 men in normal working activities, without clinical evidence of cardiovascular or bronchopulmonary disease. Office workers and labourers (industrial) were about equal in number. On physical examinations and in laboratory tests including examinations of the bellows function of the lungs and electrocardiograms, none of these subjects demonstrated cardiovascular or respiratory disease. The selection of presumably healthy individuals as normal subjects or controls is difficult, since subjects may suffer from various degrees of clinically silent atherosclerosis.

The average age of the control subjects was 49.4 years, ranging from 39 to 62 years. Their heights and weights are shown in Table I.

Table I Height and weight in control series (No = 31)

	Weight in kg	Height in cm
M	73.5	175.2
S D	6.22	6.74
Range	64—84	161—187

VC varied between 3,870 and 5,990 ml (BTPS) with a mean of 4,845 (S D ± 546). Mean value for VC expressed as percentage of predicted normal was 108%. FEV$_{1.0}$ expressed as percentage of VC varied between 70 and 89% with a mean of 78 (S D ± 5.3).

Patient series

In Oslo patients with myocardial infarction are referred to several medical departments at random. The present investigation is based on patients from Ullevål Hospital, Medical Department VIII.

The diagnostic criteria for myocardial infarction have been

1. Pain of typical character and localization.
2. The development of an abnormally deep Q-wave and RS-T elevation (not previously registered) in a 12 lead electrocardiogram (the standard leads I, II, and III, unipolar leads aVR, aVL, and aVF and V_{1-6}.)
3. Laboratory examinations including at least two of the following criteria
   a. A rise in body temperature.
   b. Leucocyte count > 10,000 per mm³
   c. Increase in erythrocyte sedimentation rate up to at least 15 mm per hour
   d. Rise in serum glutamatic-oxalacetic transaminase (SGOT) to 40 units or more.

The author was not involved in the diagnostic evaluation.

During the 3-year period from September 1962 to September 1965, a total of 146 men, 64 years of age or below were discharged from Medical Department VIII after being treated for their first myocardial infarction. Of these, 95 patients were included in the present investigation, while 51 patients with anamnestic evidence of other diseases assumed to interfere with the evaluation of the cardio-pulmonary response to muscular exercise were excluded for the following reasons. 15 patients had other heart diseases, 10 had lung diseases, and 3 had blood diseases. Immobilising muscular/skeletal disturbances made the examination impossible in 9 patients, and 9 patients were excluded because of severe psychiatric disturbances. In addition, 5 patients were either unwilling to be tested or were not invited.

Table 4.3 Means and S.E.M. of self-rated psychological variables for male and female groups and for group differences (uneasiness, agitation and expectancy), and tests of significance between periods and between group means.

Variable	Period	Male group (M) Mean ±	S.E.M.	Female group (F) Mean ±	S.E.M.	Diff. betw. gr. (M − F) Mean ±	S.E.M.
Uneasiness	A	1.31	0.10	1.81	0.18	0.50*	0.21
Males, N = 48	B	1.56	0.13	1.91	0.16	−0.35ns	0.21
Females, N = 51	C	1.15	0.07	1.40	0.12	−0.[illegible]5ns	0.14
	$B - \frac{A+C}{2}$	0.33	0.11	0.30ns	0.18	0.03ns	0.22
	A − C	0.16ns	0.10	0.41	0.19	−0.25ns	0.22
Agitation	A	1.13	0.06	1.23	0.09	−0.10ns	0.11
Males, N = 47	B	2.59	0.23	2.96	0.22	−0.37ns	0.32
Females, N = 52	C	1.27	0.09	1.42	0.12	−0.15ns	0.15
	$B - \frac{A+C}{2}$	1.39***	0.23	1.63	0.20	−0.[illegible]4ns	0.30
	A − C	−0.14ns	0.10	−0.19ns	0.14	0.05ns	0.18
Expectancy	A	3.44	0.22	2.73	0.21	0.71	0.31
Males, N = 48	B	4.00	0.20	2.57	0.23	1.43	0.30
Females, N = 51	C	1.29	0.09	1.40	0.12	−0.11ns	0.15
	$B - \frac{A+C}{2}$	1.63	0.17	0.50**	0.18	1.13	0.25
	A − C	2.15	0.25	1.33**	0.23	0.82	0.34

The ratings were made on 6-point scales ranging between "much" (6 points) and "not at all" (1 point). *A* stands for the period 0−1 1/2 hours, *B*, 1 1/2−3 hours, and *C* 3−4 1/2 hours.

crease in frequency while at the extreme of sexual excitation we find a *few* females but no males. The mean *level* of sexual arousal is significantly higher in the male group compared with the female during the film period but not during the control periods. This sex difference also applies to the *increase* in sexual arousal that is reported to have occurred during the film showing as compared with the control periods (table 4.2).

4.4.1.2 Pleasurable sensations

Both groups reported a moderate significant increase in pleasurable sensations during the film period, the increases as well as the mean level reached being significantly higher in the male group (table 4.2).

4.4.1.3 Unpleasant sensations

A significant increase in "unpleasant sensations" was also reported by both sexes during the film period, the mean level but not the change being significantly higher in the female group. It may be noted that the absolute ratings were relatively low in both groups (table 4.).

4.4.1.4 General emotional arousal

In their overall assessment of "emotional arousal" whatever its quality both groups reported significant but similar increases during the film period, the female group also being slightly but significantly more aroused during the first control period, i.e. prior to the film (table 4.[illegible]).

4.4.1.5 Uneasiness agitation

The mean questionnaire scores of "uneasiness" increased very moderately in both sexes during the film period, the increase reaching statistical significance in the male but not in the female group (table 4.3). This is a reflection of the fact that the mean questionnaire score was significantly higher in the female group during the first control period, i.e. before the start of the film period. There were no statistically significant dif

Table 4 4 Means and S E.M. for male and female groups and for group differences in catecholamine excretion, creatinine excretion, urine flow and specific gravity Tests of significance between periods and between groups.

Variable	Period	Male group Mean ±	S.E.M.	Female group Mean ±	S.E.M	Diff betw gr (M−F) Mean ±	S.E.M.
Adrenaline ng min	A	8.67	0.55	5.50	0.31	3.17	0 62
	B	14.37	1.57	6 09	0.51	8.28	1 63
	C	5 69	0.68	3 86	0.33	1.83	0.75
	$B-\frac{A+C}{2}$	7 19*	1 15	1 41	0.28	5 78	1 18
	A−C	2.98**	0 47	1 64	0.26	1.34	0.53
Noradrenaline ng min	A	24.96	1 08	22.71	1 09	2.25ns	1.52
	B	35 68	1 62	29.57	1 73	6.11	2.34
	C	28.28	1.26	22.39	1.36	5.89*	1.83
	$B-\frac{A+C}{2}$	9.06	0.94	7.02*	0.94	2.04ns	1 31
	A−C	−3.31	0.76	0.32ns	0.73	−3 63	1 04
Urinary creatinine mg min	A	1.25	0.03	0.88	0 02	0.37*	0 03
	B	1.29	0 04	0.82	0.03	0.47	0.04
	C	1.33	0.06	0.82	0.03	0.51	0.05
	$B-\frac{A+C}{2}$	0.00ns	0.03	−0 03ns	0.03	0 03ns	0 04
	A−C	−0.08ns	0.05	0.06	0 03	−0 14	0.05
Urine volume ml min	A	1.20	0.10	1 66	0.18	−0.46	0.20
	B	1 70	0.15	2.25	0 19	−0.55	0.24
	C	0.80	0.05	0 71	0.07	0.09ns	0.09
	$B-\frac{A+C}{2}$	0 70*	0.13	1 06	0 15	−0.36ns	0.20
	A−C	0 40*	0.10	0.95	0.17	−0 55	0.19
Specific gravity (N−1) × 1000	A	20.96	0.99	12.80	1 19	8 16	1 49
	B	15.37	1 03	8 69	1.00	6.68	1.40
	C	22.02	0.76	18.50	0.94	3.52	1 16
	$B-\frac{A+C}{2}$	−6.12*	0.73	−6 96	0.91	0.84ns	1 12
	A−C	−1.06ns	0.92	−5 70*	1.28	4 64	1 51

F[illegible] ach determination except the urinary creatinine levels (30 males and [illegible]6 females), there were 46 males and 4[illegible] [illegible]males being tested.
[illegible]ands for the period 0−1 1/2 hours *B*, 1 1/2−3 hours, and *C* 3−4 1/2 hours.

ferences between the groups as to changes in questionnaire scores during the experiment.

The questionnaire scores for agitation increased slightly but significantly during the film period, similarly in both groups. No significant differences were found between the groups (table 4 3)

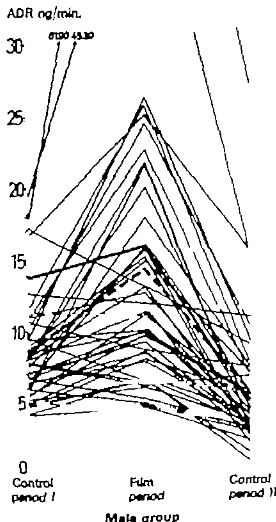
4 4.1.6 Expectancy

The mean "expectancy" scores were significantly higher in the males prior to, as well as during, the film period. Expectancy increased significantly in both groups during the film period, the increase, however being significantly more pronounced in the male group (table 4 3)

4.4.2 Physiological variables

4 4.2.1 Adrenaline excretion

As shown in table 4 4 and figure 4 [illegible], the adrenaline excretion of the male group increased markedly and significantly during the film period. The corresponding increase in the female group, although reaching the same level of significance, was markedly and significantly smaller Both sex



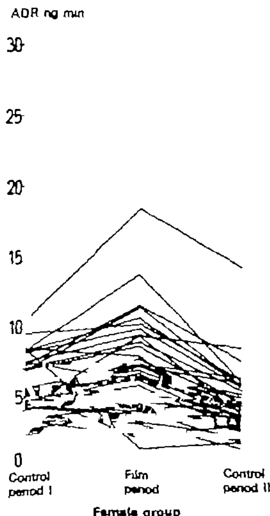


Figure 4 2. Individual values for urinary adrenaline excretion in males (left) and in females (right) during visual sexual stimulation and during control conditions. Dashed line indicates mean values.

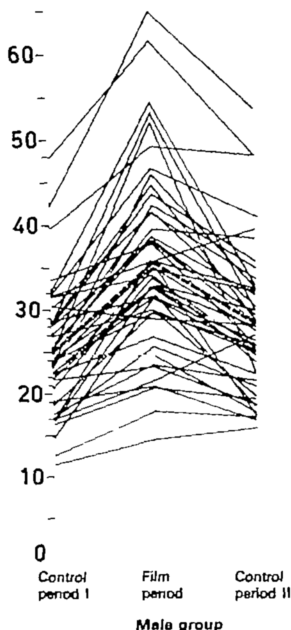
es displayed a significant fall from the first to the second control period. The excretion levels of the males were significantly higher than those of the females throughout the experiment.

4 4.2 2 Noradrenaline excretion

Table 4 4 and figure 4 3 demonstrate that noradrenaline excretion increased markedly and significantly during the film period, the reaction being of the same magnitude in both groups. During the second control period, the noradrenaline excretion of the female but not of the male group returned to the level in the first control period. During the film period and during the second control period, the excretion level was significantly higher in the male group.

4 4.2.3 Urinary creatinine

The urinary creatinine excretion of the male group did not change significantly during the experiment. The female group, however showed a significant decrease in creatinine excretion from the first to the second control period. The difference between the groups is significant in this respect. Throughout the experiment, the creatinine excretion of the female group was lower than that of the male (table 4 4).



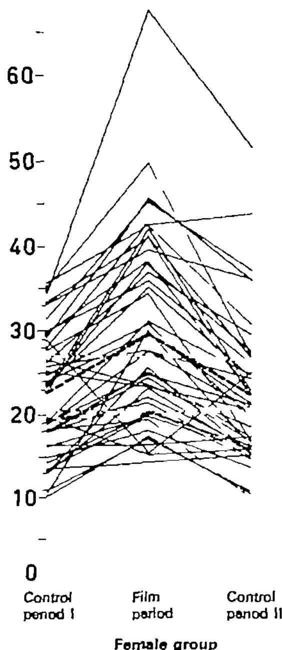
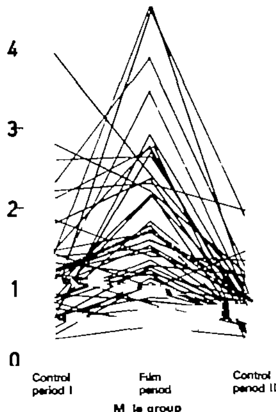


Figure 4.3 Individual values for urinary noradrenaline excretion in males (left) and in females (right) during visual sexual stimulation and during control conditions. Dashed line indicates mean values.

4.4.2.4 Urine flow

There was a marked and significant increase in urine flow during the film period, similar in both groups, and a marked and significant decrease from the first to the second control period, this being significantly more pronounced in the female group (table 4.4 and figure 4.4). During the first two periods, the urine flow was significantly higher in the female group.



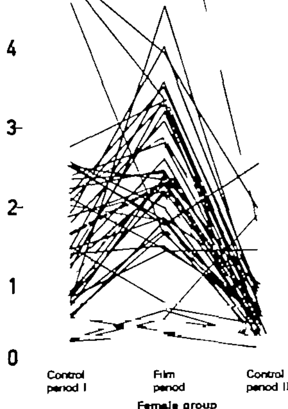


Figure 4·4 Individual alues for urine flow in males (left) and females (right) during visual sexual stimulation and during control conditions. Dashed line indicates mean alues.

4 4.2.5 Specific gravity

Urinary specific gravity decreased significantly and markedly during the film period, to a similar extent in both sexes. In the male group the increase in specific gra ity from the first to the second control period was slight and nonsignificant. In the female group, however, there was a pronounced and highly significant increase, which was significantly higher than that of the male group. During the first two periods of the experiment, specific gravity was significantly lower in the female group than in the male (table 4 4 and figure 4.5).

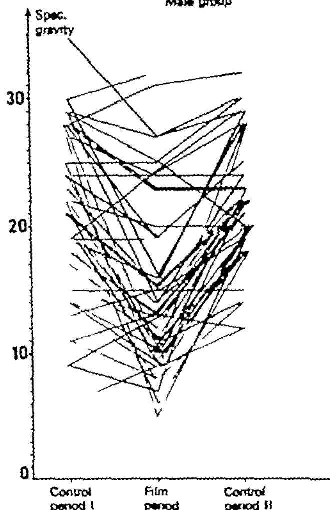
4.5 Discussion

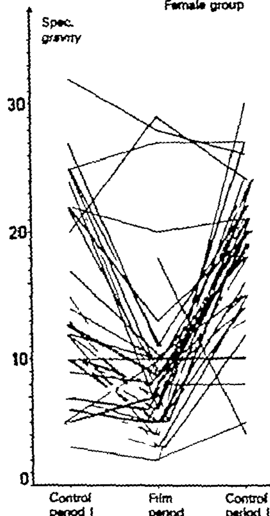
4.5.1 Sexual arousal and urinary excretion of adrenaline and noradrenaline

During the film period there was a significant rise in the urinary excretion of adrenaline as well as of noradrenaline in both sexes, probably reflecting an increase in sympathoadrenomedullary function (Euler 1964).

The question is whether this rise is ascribable to an increase in sexual arousal. A number of alternative explanations should be considered.

Firstly it might be argued that the catecholamine increases were a response, not to the





F gur 4 5 Indn idual values for urinary pecific gra ity in males (left) and females (right) during visual sexual stimulation and during control conditions. Dashed line indicates mean values.

specific qualities of the films shown, but to the cinematographic experimental situation as such.

However the results of an earlier experiment of similar design do not support this explanation (cf Chapte 3) Bland natural-scenery films were hown to cause a significant *decrease* in the catecholamine excretion of healthy females. Thus, the *ne ease* found in the sex film experiment were p obably not due to an unspecific influence of the cinematographic situation as such but to the emotional elements present in the film program.

Secondly it is of course impossible to design a film program in such a way that sexua is the only reaction to it in all subjects. Con sequently it may be argued that the changes in catecholamine excretion during the film period chiefly reflect emotional reactions other than the sexual ones. The film program, for instance, may have induced feelings of uneasiness. As seen from tables 4 2 and 4.3 such reactions were, indeed, reported to occur during the film period. How ever if the catecholamine reactions are to be ex plained in terms of an emotional arousal of a predominantly non-sexual character why did the male group react *with a significantly stronger* adrenal than the female one, in spite of the aw significant intergroup dif

ferences as to self-rated and reported non-sexual emotional reactions speak in favour of somewhat stronger unpleasurable feelings in the female group? There is no a priori reason to believe that the non-sexual reactions of the females—contrary to their report—were less pronounced than those of the males.

It is therefore considered more probable that the sexual arousal evoked by the experimental stimulus is, indeed, the main factor related to the increase in catecholamine excretion in both groups during the film showing.

The decrease in adrenaline excretion in both groups from the first to the second control period is probably at least in part due to diurnal variation (Levi, 1968).

4.5.2 Relationship between sexual arousal and sympathoadrenomedullary activity

For reasons mentioned in the introduction, our next problem concerns the *quantitative* relationship, if any between sexual arousal and sympathoadrenomedullary activity Needless to say neither of these functions was accessible for direct measurement. The psychological reactions were presumably of a composite character and probably difficult to report correctly even for the rather sophisticated subjects participating in this study Furthermore, such reports are bound to be influenced by the well known disinclination or even disability of a number of people honestly to report reactions that are usually taboo or considered to be socially controversial. Finally for reasons mentioned earlier the subjects knew to what type of stimuli they were to be exposed and may have presumed that they were expected to react with sexual arousal. This cognitive factor may have contributed to this type of reaction at the expense of other types that were possibly experienced but perhaps less readily reported by the subjects. Accordingly there is good reason why the subjects self-ratings cannot be taken as a true index of what they actually experienced, and still less, of the total sexual arousal of the organism.

Neither can it be taken for granted that the urinary excretion of free catecholamines is a simple function of the total release of these hormones during the period of urine collection (cf paragraph 2.14) even if this release is probably the major determinant of the amount excreted.

Against this background, it seemed unlikely that the correlation between these two sets of variables would be very high. Taking the correlation in the *female* group between the simultaneous changes in catecholamine excretion and sexual arousal during the film period in relation to the mean of the two control periods (B-[(A+C) + 2]), we found that r = 0.27 for adrenaline as well as for noradrenaline ($0.05 < p < 0.10$) The corresponding correlation coefficients were also calculated for the changes from the first to the second control period (A—C) Here, the product-moment correlation in the *female* group between sexual arousal and adrenaline was 0.34 ($p < 0.05$) and between sexual arousal and noradrenaline 0.38 ($p < 0.01$) The corresponding correlation coefficients for the changes from the first control period to the film period (B—A) were 0.38 ($p < 0.01$) and 0.29 ($0.05 < p < 0.10$) respectively

The corresponding correlation coefficients in the *male* group were considerably lower namely 0.11 0.06, −0.16, 0.29 0.13 and 0.13 respectively ($p > 0.05$), cf table 4 5 The reason for this sex difference is not known. It is possible that the females gave more honest and reliable reports of their reactions than did the males. This would be in accord with the findings of Maslow (1965), indicating that "females were far more honest and open and found it easier to talk about these things because their self-esteem is less involved" Of course, this need not be the only or even the main explanation. The matter no doubt deserves further study

4.5.3 Sex differences in the reactions to visual sexual stimulation

Our data indicate that some individual females actually were more responsive to the stimuli used than any of the men in the sample, both with respect to psychological and to noradrenaline reactions. In general, however the female group

Table 4 5 Correlation coefficients (r) between simultaneous changes in catecholamine excretion and self rated sexual arousal in male and female groups.

Variable	Group	SEXUAL AROUSAL		
		$B-\frac{A+C}{2}$	A−C	B−A
Adrenaline	Males	0.11ns	0.16ns	0.13ns
	Females	0.27(*)	0.34	0.38
Noradrenaline	Males	0.06ns	0.29(*)	0.13ns
	Females	0.27(*)	0.38	0.29(*)

A stands for the period 0−1 1/2 hours *B* for 1 1/2−3 hours, and *C* for 3−4 1/2 hours.
(*) = $0.05 < p < 0.10$

reacted less than the male group both with respect to reported sexual arousal (figure 4 1 and table 4 2) and to adrenaline excretion (figure 4 2 and table 4 4)

This might be explained by assuming that in females sexual arousal is not—as in males—related to the function of the sympathoadrenomedullary system. However the fact that the catecholamine changes in the female group are positively and significantly correlated to the changes in sexual arousal runs counter to such an assumption

A number of other possible or probable explanations of the sex difference in catecholamine excretion remain to be discussed. One hypothesis is that females are adrenomedullarily *less reactive* at least in response to psychological stimulation. Such a female adrenomedullary "hyporeactivity" has, *indeed, been demonstrated in response to* mental work (Frankenhaeuser 1972) True both sexes have been shown to excrete similar amounts of 3-methoxy-4-hydroxy mandelic acid—one of the major catecholamine metabolites—in response to stressor situations of a more potent type and with no performance involved, like exposure to cardiac catheterization (Schmid et al., 1965) and to dental treatment (Weiss et al., 1965) This, however does not necessarily invalidate the hyporeactivity hypothesis

A further possibility is that most females were not markedly aroused by the sex film program because the stimulus situation lacked "romantic" elements. Such elements, however were abundantly present in a study of similar design (Levi, 1967) In this study 15 of the subjects who took part in the study reported in Chapter 3 were shown a program considered to be highly romantic and sensual, composed of love scenes from e.g. Dassin's "Fedra" Thomas "The Wild and the Willing" Valcroze's "Le Coeur Battant" Schlesinger's "A Kind of Loving" and Malle's "Les Amants" The reason for selecting relatively short scenes from nine different films instead of choosing one complete normal-length film was the author's inability to find any single film that fulfilled the criteria of being exclusively characterized by joyous love and reasonably free from other elements such as tragedy comedy and drama.

Briefly this program proved still less effective in eliciting catecholamine responses in the group of 15 female office-clerks. However this does *not necessarily invalidate the explanation under* discussion, because each of the nine film sequences might have been too short to allow identification and to provoke sexual arousal, or because what is needed to provoke such arousal in many females is the *combination* of "sexual and "romantic" elements and not each element per se.

The sex differences might also be explained by arguing that the films were of a kind that *primarily appeals to a male audience, in that the* possibilities for identification are greater However the films used depicted the participation of both sexes to the same extent in a number of heterosexual activities, including undressing, nudity petting, fellatio, cunilingus and coitus.

Thus, the identification possibilities were presumably much the same for subjects of both sexes.

Other hypothetical explanations of the sex difference with respect to changes in catecholamine excretion may include the factors of body weight and age (cf. paragraph 2.10). It has been shown, however, that the first of these factors is not significantly correlated to catecholamine excretion (Bergsman, 1959), while no significant difference existed between our two groups as regards the other.

The difference in sexual experience between the two groups, as presented in table 4.1, might also have contributed to this difference in reaction if one assumes that previous sexual experience conditions an individual to subsequent reactions to visual sexual stimuli. This assumption, however, is not supported by the results of a statistical comparison between the 31 "experienced" and 14 "inexperienced" female subjects as to changes (B-[(A+C) + 2]) in sexual arousal and adrenaline excretion, the means and standard errors of the means being 3.6 ± 0.6 and 1.84 ± 0.41 for the "experienced" group and 3.2 ± 0.6 and 1.01 ± 0.40 for the "inexperienced" group, respectively ($p > 0.05$).

Thus, although *no firm conclusions* can be drawn, the author is inclined to interpret the present results as favouring Kinsey's hypothesis that females are generally less aroused than males in response to *visual* sexual stimulation (Dengrove, 1967; Sigusch et al., 1970).

4.5.4 Sexual arousal and renal function

Both sexes displayed a pronounced increase in urine volume during the film period as compared with the control periods, and a corresponding decrease in specific gravity. These changes cannot be ascribed to circadian variation or the hydration procedure, because a similarly designed control experiment without exposure to any experimental stimuli showed a steady decline in diuresis over time (Levi, 1968). Thus it seems reasonable to interpret the changes as being due to the stimuli presented in this experiment, i.e. the sex films. On the other hand, in another similarly designed study (cf. Chapter 3) an increase in diuresis and a decrease in specific gravity were demonstrated in response to a number of other kinds of film programs.

Thus, the psychogenic diuresis (Miles et al., 1952) demonstrated in the film experiments (reported in Chapter 3) occurred in the present study as well (cf. table 4.4).

The urine flow of the females was higher and the specific gravity lower than that of the males. This group difference is probably primarily a reflection of the females' significantly lower body weight (female and male means and S.E.M. being 56.8 ± 0.7 kg and 72.6 ± 0.8 kg, respectively) and consequently their higher intake of fluid/kg body weight.

However, one still has to explain the sex differences in *antidiuretic* reaction from the first to the second control period, when the decrease in urine flow and the increase in specific gravity were significantly more marked in the female group *in spite of* the higher degree of hydration. This might theoretically be due, in part, to a combined effect on renal haemodynamics of the catecholamines—which is different at different levels (cf. paragraph 2.13)—and of the antidiuretic hormone, the release of which is probably elevated during sexual excitation (Eränkö et al., 1953; Friberg, 1953).

The change in creatinine excretion between the two control periods is also significantly different in the two sexes, the males exhibiting a nonsignificant increase and the females a small but significant decrease, probably reflecting corresponding changes in glomerular filtration.

4.5.5 Non-sexual psychological reactions

The males' slightly higher scores of pleasurable feelings and slightly lower scores of unpleasurable ones are in accordance with the Kinsey hypothesis that men are more prone to sexual arousal from visual stimuli than women, there being a significant positive correlation between sexual arousal and pleasurability ratings (males: $r = 0.40$, $p < 0.05$; females: $r = 0.70$, $p < 0.01$). The higher expectancy ratings in the male group probably reflect the same general trend. Ratings of uneasi-

ness, on the other hand, were generally very similar in both groups with respect to levels as well as to changes in levels. This runs counter to the common assumption that young females are disturbed more easily by the type of stimuli applied here than young males.

4 6 Summary

A total of 53 female and 50 male students were shown a 1 1/2 hour film program (comprising four short, silent, sexual films) preceded and followed by control periods of equal duration. Adrenaline and noradrenaline excretion increased significantly in both groups during the film period in relation to control levels before and after. During the film period, significant increases in sexual arousal were reported by both sexes, the self rating scores as well as their increases, however being significantly higher in the male group. This difference in reported sexual arousal was paralleled by a corresponding difference in the urinary excretion of adrenaline, both the excretion levels and the increases over the control levels being significantly higher in the males. Possible explanations for the sex differences are discussed. Changes that occurred in urine flow specific gravity and creatinine excretion during and after this type of psychosexual stimulation are reported, as are some psychoendocrine relations. Their possible significance is discussed against the background of the Kinsey hypothesis that men are more prone than women to sexual arousal from visual stimuli.

4 7 Acknowledgements

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5 STRESSOR INDUCED CHANGES IN PLASMA LIPIDS AND URINARY EXCRETION OF CATECHOLAMINES AND THEIR MODIFICATION BY NICOTINIC ACID

By Lars A. Carlson, Lennart Levi and Lars Orb

5.1 The problem

Chronic exposure of man to various psychosocial stimuli leads to increased plasma concentrations of cholesterol (Friedman et al., 1958 Thomas and Murphy 1958 Wertlake et al., 1958 Grundy and Griffin, 1959 a) and very low density lipoproteins (Grundy and Griffin, 1959 b) Little is known about the *mechanism* of this psychosocially induced rise in plasma lipoproteins. The relevant data may be summarized as follows.

Distress reactions are accompanied by a considerable increase in the urinary excretion of adrenaline and noradrenaline, probably reflecting a corresponding release of catecholamines from the sympathoadrenomedullary system (cf. Levi, 1961 Euler 1964 Levi, 1967 a, Levi, 1967 b). Catecholamines stimulate the mobilization of free fatty acids (FFA) from adipose tissue (cf. Havel and Goldfien, 1959 Carlson et al., 1965) Short term emotional arousal in man, such as fear anxiety and psychosexual stimulation, has also been found to be accompanied by increased plasma levels of FFA (Bogdonoff et al., 1959 Cardon and Gordon, 1959· Bogdonoff et al., 1960 a). Plasma FFA are rapidly incorporated into triglycerides of the liver and of the plasma lipoproteins (cf. Carlson et al., 1965 a). The liver is probably the main site of formation of endogenous plasma lipoproteins (cf Carlson et al., 1965) In the fasting state, when lipogenesis is low FFA are the main precursors of the hepatic and plasma triglyceride fatty acids. An increased mobilization of FFA from adipose tissue, e.g. by catecholamines, leads to a rise in the triglyceride content of the liver (Friedman and Byers, 1960 Feigelson et al., 1961 Carlson and Liljedahl, 1963 Carlson et al., 1965 b) and plasma (Friedman and Byers, 1960 Carlson et al., 1965 b) Prolonged treatment with catecholamines also leads to hyperlipoproteinemia.

These data suggest the following *hypothesis* for the mechanism underlying the hyperlipoproteinemia induced by psychosocial stimuli. Exposure to such stimuli can evoke an increase, mainly mediated by the sympathoadrenomedullary system, in the mobilization of FFA from adipose tissue. As a result, the amount of triglycerides in the liver increases, thereby stimulating the secretion into plasma of triglycerides from this organ. Ultimately plasma triglyceride levels rise, as do the levels of other lipoprotein constituents, e.g. cholesterol.

5.2 Choice of methodology

One way of testing this hypothesis would be to expose subjects to psychosocial stimuli of relatively short duration and moderate intensity (Levi, 1961), to make sure that this exposure does, indeed, evoke distress reactions, and to study the effect, if any on catecholamine excretion [as an index of sympathoadrenomedullary activity cf. Euler (1964)] and on the concentration in arterial plasma of FFA, triglycerides, and cholesterol. In order to assess the role of FFA mobilization in this context, the effect of an antilipolytic agent (nicotinic acid) on psychosocially induced responses of the plasma lipids might be studied, as nicotinic acid is known to inhibit the stimulated FFA mobilization produced by exogenous catecholamines in vivo (Carlson and Orö, 1962 Carlson et al., 1963) and in vitro (Carlson, 1963 a, Carlson, 1965).

To accomplish this, we needed a set of psychosocial stimuli that could be assumed to be

Table 5.3 Self-rated distress during the second 2-hour period, involving rest for the control group but "work" conditions for the other two groups.

	Control group	Untreated stress gr	Treated stress gr
The most unpleasant experience I ever had (det obehagligaste jag varit med om)	0	0	0
Very pressing and unpleasant (mycket pressande och obehagligt)	0	2	0
Rather pressing and unpleasant (ganska pressande och obehagligt)	2	3	1
Rather trying but not actually unpleasant (ganska besvärligt men inte direkt obehagligt)	0	6	7
It did not bother me on the whole (Jag var på det hela taget oberörd)	9	0	3
Total	11	11	11

artery: heparin was not used. The subjects were then allowed to rest for at least 1 hour i.e. until 9 a.m., when the experiment proper started. The experimental procedure involved three consecutive 2-hour periods, the second being the "*work*" period for two of the groups, as indicated above. As indicated above, the task involved sorting small shiny steel balls of four very similar sizes in the presence of a loud industrial noise, variations in the intensity of a dazzling light, rush due to lack of time, and standardized criticism. The criticism was presented in writing 13 and 28 minutes after the beginning of the "work" period, the subjects being blamed for slowness and carelessness, respectively at these times. During the *control* periods before and after this period (i.e. during the first and third 2 hour period) the subjects relaxed by reading a weekly magazine and listening to soft music. No food was served. At the beginning of each period, the subjects ingested 300 ml of tap water. Urine samples were collected at the end of each 2 hour period.

Serial blood samples were drawn into heparinized syringes every 15th or 30th min (figure 5 1

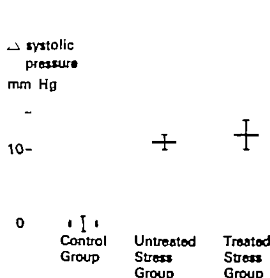


Figure 5.1 Mean ± standard error of the mean for the changes in systolic blood pressure during the first 15 minutes of the second 2-hour period in the control group (left), the untreated stressor-exposed group (centre) and the treated stressor-exposed group (right).

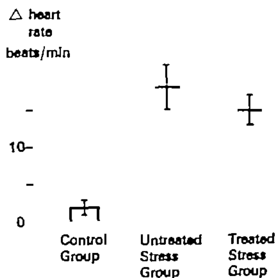


Figure 5.2. Mean ± standard error of the mean for the changes in heart rate during the first 15 minutes of the second 2-hour period in the control group (left), the untreated stressor-exposed group (centre) and the treated stressor-exposed group (right).

Table 5 4 Statistical comparison of means ± SEM and changes in urine volume, specific gravity urinary creatinine, systolic and diastolic blood pressure and heart rate in the three groups

Group	Period	Urine volume ml/min	Spec. gra. (N 1	Creatinine 1000 mg/min	Syst. blood pressure mm Hg	Diast. blood pressure mm Hg	Heart rate beats/min
Controls	A	3.10 ± 0.5	8.0 ± 1.3	1.14 ± 0.10	147.6 ± 2.0	90.3 ± 4.4	65.8 ± 2.0
	B	2.24 ± 0 6	8 ± 1.1	1.02 ± 0.05	138.4 ± 7.4	86.4 ± 4.5	65.1 ± 1.8
	C	1.79 ± 0.18	8.8 ± 1.4	1.08 ± 0.06	138.0 ± 8.2	84.3 ± 6.	65.1 ± 2.8
	B−A	−0.86	0	−0.13	− 9.2*	− 3.9*	− 0.7
	B−C	−0.45	−1.0	−0.07	+ 0.4	− 2.1	± 0.0
	A−C	−1.37*	−0.8	−0.06	+ 9.6	− 6.0	− 0.7
Untreated Stress	A	3.10 ± 0.41	8.6 ± 2.0	0.90 ± 0.06	158.8 ± 8.7	98.7 ± 5.2	62.7 ± 3.2
	B	1.53 ± 0.27	11.6 ± 1.8	0.86 ± 0.05	153.8 ± 8.3	99.1 ± 5.6	76.6 ± 5.1
	C	2.18 ± 0.79	8.4 ± 2.	1.03 ± 0.15	156.3 ± 7.4	98.9 ± 4.7	64.9 ± 3.8
	B−A	−1.56 **	−3.0	−0.04	− 5.0	− 0.4	−13.8***
	B−C	−0.65	3.	−0.17	− 2.4	+ 0.2	−11.6
	A−C	−0.93	−0.2	−0.13	− 2.6	− 0.2	− 2.2
Treated Stress	A	3.75 ± 0.40	7.9 ± 1.9	1.19 ± 0.06	143.4 ± 4.8	93.6 ± 2.9	69.4 ± 3.3
	B	2.61 ± 0.45	9.8 ± 2.0	1.04 ± 0.06	144.4 ± 4.4	95.9 ± 2.3	79.3 ± 3.1
	C	1.64 ± 0.30	12.4 ± 2.5	0.99 ± 0.06	141.6 ± 4.0	95.9 ± 2.9	69.2 ± 2.8
	B−A	1.15	−1.9	−0.14	+ 1.0	− 2.3	+ 9.8*
	B−C	−0.97*	−2.6	+0.05	+ 2.7	± 0.0	+10.1**
	A−C	−2.11	−4.5	+0.20*	− 1.7	− 2.3	− 0.3
Group differences Untreated Stress minus Controls	A	±0.00	+0.6	−0.24	+11.2	− 8.4	− 3.1
	B	−0.71	+3.8	−0.13	+15.4	−12.7	−11.4
	C	−0.40	−0.4	−0.05	−18.3	−14.6	− 0.2
	B−A	−0.71	+3.2	+0.09	+ 4.2	− 4.3	−14.6 **
	B−C	−1.10**	+4.2*	−0.11	− 2.9	− 1.9	−11.6**
	A−C	−0.40	+1.0	−0.19	− 7.1	− 6.2	− 2.9
Treated Stress minus Controls	A	−0.65	−0.1	+0.04	− 4.3	+ 3.3	− 3.6
	B	+0.37	+2.0	+0.03	− 5.9	+ 9.6	−14.2***
	C	−0.14	+3.6	−0.09	+ 3.6	+11.6	+ 4.1
	B−A	−0.29	+2.1	−0.02	+10.2**	+ 6.	+10.6***
	B−C	+0.51	−1.6	+0.12	+12.3	− 2.1	+10.1 **
	A−C	+0.80	−3.6	+0.14	− 7.9	− 8.3	− 0.4
Untreated Stress minus Treated Stress	A	−0.66	+0.7	−0.29**	+15.4	+ 5.1	− 6.7
	B	−1.08	+1.8	−0.18	+ 9.4	+ 3.2	− 2.7
	C	+0.54	−4.0	+0.05	+14.6	+ 3.0	− 4.3
	B−A	−0.42	+1.1	+0.10	− 6.0	− 1.9	+ 4.0
	B−C	−1.62*	+5.7	−0.23	− 5.2	− 0.2	+ 1.6
	A−C	−1.20*	+4.6	−0.33	+ 0.8	+ 2.1	− 2.4

A stands for the period from 0–2 hours, *B* for 2–4 hours, and *C* for 4–6 hours.

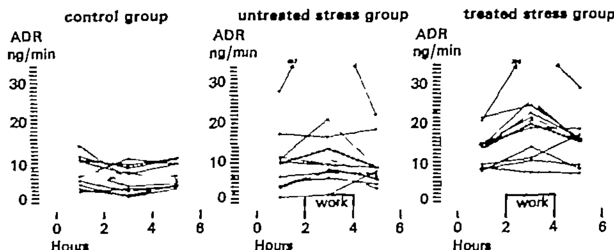
and table 5 4) immediately centrifuged, and the plasma lipids were extracted. FFA were deter mined by the method of Dole (1956) as modified by Trout et al (1960), total cholesterol according to Sperry and Webb (1950) and Carlson (1960), and triglycerides by the method of Carlson (1960 and 1963 b)

During the first 2-hour period, the subjects were asked how they felt now immediately before the start of the test" After the second -hour period, the subjects were asked how they felt during the test During this period observations were made every 10 minutes by one of the authors concerning the "level of general emotional arousal" manifested by the subjects.

5 4 Results

5.4.1 The pre-experimental period: changes in plasma triglycerides and cholesterol

As indicated earlier the subjects were assigned to the three groups so that means and variations with respect to blood lipid levels, i.e. the levels found during the screening procedure 3—8 wee



Figur 5.3 Individual values for the excretion of adrenaline during the three 2-hour periods in the control group (left), the untreated stressor-exposed group (centre) and the treated stressor-exposed group (right). Dashed line indicates means. Arrows indicate nicotinic acid administration (0.5 g 6 times, i.e. every 30 minutes in the treated stressor-exposed group).

before the actual experiment were similar in all groups (table 5) In the interval between this measurement and the actual experiment i.e. dur ing the pre-experimental period, the triglyceride levels of the *control group* fell moderately but significantly In the *untreated stress group* there was no significant change. The reason for this difference in reaction pattern is not known but may ha e been due, at least in part, to a hy pothetical, relative decrease in apprehension in the control group, in contrast to some similarly hypothetical, moderate but continuous apprehension in the untreated stress group, as both groups knew in advance the experimental procedure they were to undergo. The influence on the tri glyceride levels may have been similar to that found in the experiment proper as indicated below Finally the *treated stress group* showed a significant and pronounced fall in plasma triglycerides during the pre-experimental period, probably attributable to the remaining lipid-lowering effect of the *p t eatment* with nicotinic acid (Altschul et al 1955)

5.4.2 The experiment proper

5 4.2.1 Some indices of dist ess reactions

During the first -hour (control) period, eight, eight and ten of the subjects in the control group, the untreated and the treated stress groups, re spectively reported that they felt calm and unconcerned" generally in support of our assumption that this period would be characterized by feelings of calmness and equanimity In contrast, the corresponding self ratings with regard to the second 2 hour period demonstrate moderate distress reactions in most of the subjects belonging to the untreated and treated stress groups but in only a few in the control group, see table 5.3 While performing the observations mentioned in paragraph 5.3 we found it most difficult to quantify the distress reactions, if any of our subjects on the basis of their gross behaviour Suf fice it to say that these observations, crude as they were, are generally in agreement with the data from the self ratings, indicating that both stressor-exposed groups probably experienced moderate distress during the work period whereas the control subjects during the corresponding period were relatively unconcerned.

5 4.2.2 Some indices of cardiovascular reactions

The *blood press e* (figure 5 1) of the *untreated st ess group* increased significantly when measured 15 minutes after the start of exposure to work" conditions. Systolic blood pressure increased by 1_ ±2 mm Hg and diastolic blood pressure by 9±2 mm Hg ($p < 0.001$ and $p < 0.01$ respectively) from the values found just before

Table 5 5 Statistical comparisons of mean SE ... cholesterol, and urinary adrenaline, and norа...

Group	Period[b]	Free fatty acids[a] meq liter × 10⁽		C mg ...	A	
Controls	A	78.6 ± 6	[illegible]	[illegible]	6 1 10	[illegible]
	B	79 6 ± 6 2		[illegible]	[illegible] 0 96	[illegible]
	C	82.2 ± 6 4	[illegible]	[illegible]	6 [illegible] 0 90	[illegible] 2 9
	B−A	− 1 0	0	1	− 2 04	[illegible]
	B−C	− 2.6	−1 [illegible]	− 5	− 0 98	−
	A−C	− 3 6	−0 [illegible]	− 16	1 0	− 0 9
Untreated Stress	A	71 0 ± 6 5	1 [illegible]	1 ± 13	9 53 ± 2 3	8 ± 2 8
	B	82.9 ± 6.2	1 86 0 15	278 ± 12	13 31 ± 3 94	33 1 ± 3 6
	C	93.2 ± 3 9	[illegible]00 ± 0 18	264 ± 14	8 85 ± 1 72	28.2 ± 3 8
	B−A	+11 9**	0 10	− 7	− 3 78	− 9.3**
	B−C	−10 3	−0 12	− 10	− 4 46	− 4 9
	A−C	−22.2***	−0 22	− 3	− 0 68	− 4 4
Treated Stress	A	45.2 ± 2.8	1.24 ± 0 15	199 ± 7	13.39 ± 1 39	29 0 ± 2.4
	B	27 6 ± 2.3	1 07 0 13	201 ± 9	19 52 ± 2.70	42.0 ± 3 9
	C	27 4 ± 2 1	0.99 ± 0.12	200 ± 15	14.96 ± 1 73	32.0 ± 2 6
	B−A	−17 6 **	−0.17	+ 2	− 6 13	−13 1
	B−C	− 0 2	+0.08	+ 1	− 4.57	−10.0*
	A−C	−17 7*	+0.25	− 2	− 1 57	− 3 1
Group differences	A	− 7 6	−0.55	− 37*	− 1 91	− 0.3
	B	+ 3 4	+0.66**	− 18	− 7 73	+ 9 5
Untreated Stress minus	C	+11.0	+0 78	− 23	+ 2.48	+ 3 9
Controls	B−A	−10 9*	+0 12	+ 19*	− 5.82*	− 9 2
	B−C	− 7.6	−0 12	+ 5	+ 5 45	− 3 6
	A−C	−18.6	−0.23**	− 13	− 0.37	− 3 6
Treated Stress minus Controls	A	−33 4	±0.00	−109**	+ 5 77	− 5.5
	B	−51 9***	−0.15	− 95	−13 94***	−18 4**
	C	−54.7*	−0.23	− 91	+ 8 39***	− 7 7
	B−A	−18.6***	−0.15	+ 14	+ 8 17***	−14.9*
	B−C	+ 2.8	+0.09	− 4	+ 5.55**	−10 7***
	A−C	+21 4**	+0 23	− 18	− 2 62	− 2.2
Untreated Stress minus Treated Stress	A	+25.8**	+0.54	+ 72*	− 3.86	− 5.2
	B	+55.3	+0.81 **	+ 77**	− 6.21	− 8.9
	C	+65 7***	+1.01	+ 68*	− 6.11	− 3.8
	B−A	+29 4	+0 27*	+ 5	− 2.35	− 3 7
	B−C	−10 4	−0.20*	+ 10	− 0.10	− 5 1
	A−C	−39.9***	−0 47***	+ 5	− 2.25	− 1 4

A stand for the period from 0—2 hours, *B* for 2—4 hours, and *C* for 4—6 hours.
Mean values calculated on the basis of six measurements (period A), eight measurements (period B), or five measurements (period C) in each subject.
Mean values calculated on the basis of three measurements (period A) or two measurements (periods B and C) in each subject.

exposure started. In the *treated stress group* the corresponding increases were 14 ± 4 mm Hg and 9 ± 2 mm Hg ($p < 0.01$ and $p < 0.001$, respectively). The *control group* exhibited no corresponding change.

The experimentally induced blood pressure reactions were of short duration. Comparing the *mean* blood pressure during each of the three 2-hour periods (table 5 4), the changes between periods were not significant in either of the two work-exposed groups. The control group showed significant decreases of the mean systolic and diastolic blood pressures. The differences in levels and reactions between the two work-exposed groups were not statistically significant neither were the corresponding differences between the untreated stress group and the control group.

Exposure to the experimental work conditions

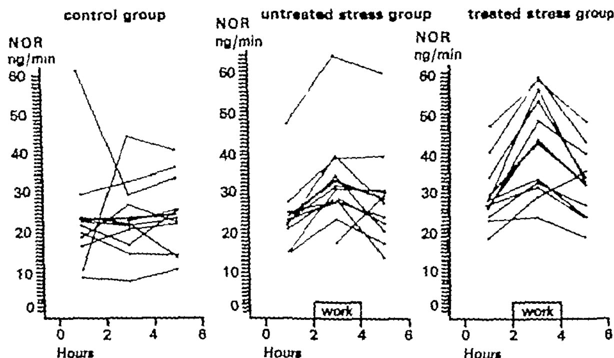


Figure 5.4 Individual values for the excretion of noradrenaline during the three 2-hour periods in the control group (left), the untreated stressor-exposed group (centre) and the treated stressor-exposed group (right). Dashed line indicates means.

was accompanied by significant accelerations in *heart rate* the levels and responses being of the same magnitude in both exposed groups (figure 5 and table 5 4) *The heart rate of the control* group remained on a constant level throughout the experiment, significantly below that during the work period for the two exposed groups.

5 4 .3 Some indices of sympathoadrenomedullary and renal reactions

In the *control group* urinary *adrenaline excretion* decreased during the second 2-hour period, although the change was not significant (table 5 5). In the *untreated stress group* there was a non significant increase during the same period, while the increase in the *treated stress group* was significant, cf figure 5 3

During the first control period, the adrenaline excretion was significantly higher in the *treated stress group* than in the *control group* (table 5 5). Otherwise there were no significant differences between the adrenaline levels of different groups during this period.

Comparing the changes in adrenaline excretion in the three groups, no significant differences were found between the two work-exposed groups, while the reactions of both groups during the *second 2 hour period differed significantly from* those displayed by the control group (table 5.5).

The *noradrenaline excretion* of the control group remained largely unchanged throughout the experiment (table 5 5) whereas in the other two groups it increased significantly and similarly during work exposure (figure 5 4) During the first hour period the level of noradrenaline excretion was much the same in all three groups.

The *urine volume* in all three groups decreased significantly between the first and the third of the three 2-hour periods. Table 5 4 lends some support to the hypothesis that both work exposure and nicotinic acid treatment affect urine flow As to urinary *specific gravity* no significant changes occurred in any group, though the general trend corresponded to that for urine flow cf table 5 4

Creatinine excretion decreased significantly in the treated stress group from the first to the third 2-hour period of the experiment (table 5 4). Apart

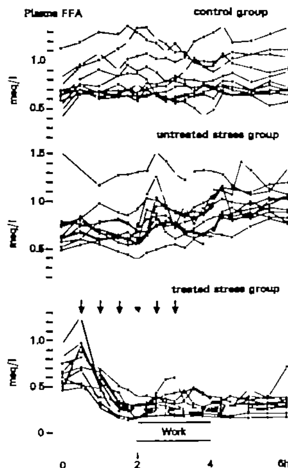


Figure 5.5 Individual values for arterial plasma levels f free fatty acids in the control group (top), the untreated stressor-exposed group (centre) and the treated stressor-exposed group (bottom). Arrows indicate nicotinic acid administration (0.5 g 6 times, i.e. every 30 minutes in the treated stressor-exposed group)

from this, there were no significant changes in any of the groups. During the first 2-hour period, when the administration of nicotinic acid started, the group receiving this exhibited a significantly higher creatinine excretion than the untreated stress group, but during the last two 2-hour periods there were no differences between the groups.

5.4.2.4 Free fatty acids triglycerides and cholesterol in arterial plasma

In the *control group* the concentration of *free fatty acids* remained largely unchanged throughout the experiment. In the *untreated stress group* FFA remained constant during the first 2 hour period and increased significantly during and after work exposure (figure 5.5 table 5.6). In the *treated stress group* the concentration of FFA fell during the first 2-hour period, when nicotinic acid treatment started (figure 5.5) reaching a mean level of 0.24±0.07 meq/liter immediately before the start of work exposure; there was no significant rise in -hour means during or after the work period (table 5.6). The absolute levels f FFA were significantly lower in the treated stress group than in the other two groups during the last two 2-hour periods.

Table 5.6 Changes in arterial concentration o FFA (in meq/l 100) from hours (just prior to start o the work period for two of the groups) and onwards

FFA concentration at 2 hours	Controls	Untreated Stress	Treated Stress
2 hours	83±8	68±7	4±7
2[illegible]	2±	1 ±3	±1
[illegible]	—3±3	24±5	5±2
[illegible]	—6±4	17±4	6±3
3[illegible]	—5±4	11±3	5±3
3[illegible]	—5±5	9±3	4±2
3[illegible]	—5±6	10±3	1±2
3[illegible]	—5±6	13±4	1±1
4[illegible]	—5±5	20±4	1±2
4	3±5	26±5	1±1
4[illegible]	9±6	21±5	1±2
5[illegible]	4±7	21±5	4±2
5[illegible]	0±8	27±5	3±2
6[illegible]	1±7	28±5	7±2*

The changes are calculated on the individual changes and are expressed as means ±S.E.M. ** and indicate that $p < 0.05$ 0.01 and 0.001 respectively for the statistical significance of the changes *from 2 hours.*

Throughout the experiment, the *triglyceride* levels of the *control group* remained unchanged, whereas those of the *untreated stress group* increased significantly towards the end, the levels being significantly higher in the latter group throughout the experiment. In the *treated stress group* on the other hand, the triglyceride level decreased progressively and significantly. The contrasting behaviour of the triglycerides in the *untreated stress group* and the *treated stress group* is summarized in figure 5.6 the group differences in respect of both the levels and the changes are highly significant throughout the study (table 5.5).

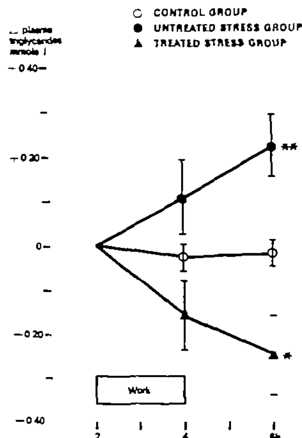


Figure 5.6 Mean ± standard error of the mean for the changes in plasma triglycerides during and after the second 2-hour period, which was designed to induce distress in the untreated and treated stressor-exposed groups but not in the control group. * and ** indicate that $p < 0.05$ and 0.01 respectively

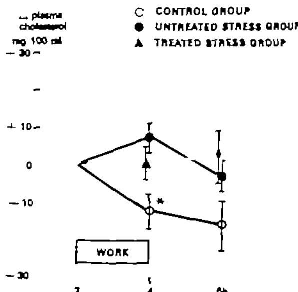


Figure 5.7 Mean ± standard error of the mean for the changes in plasma cholesterol during and after the second 2-hour period, which was designed to induce distress in the untreated and treated stressor-exposed groups but not in the control group. * indicates that $p < 0.05$

The plasma *cholesterol* levels fell slightly towards the end of the experiment in the *control group* (table 5.5 and figure 5.7) but there were no significant changes in either the *untreated stress group* or the *treated stress group*. The cholesterol levels of the treated stress group were significantly lower than those of the other groups throughout the experiment. The difference between the decrease from the first to the second 2-hour in the control group and the corresponding increase in the untreated stress group is statistically significant ($p < 0.05$) cf. table 5.5.

5.5 Discussion

5.5.1 Some general considerations

As demonstrated above the simulated work conditions provoked, in most of the subjects exposed to these conditions, (a) distress reactions, (b) cardiovascular reactions, and (c) sympathoadrenomedullary reactions. These reactions were not modified by nicotinic acid treatment, at least not to a marked degree.

Concomitantly with the reactions mentioned above, FFA and triglycerides in arterial plasma rose significantly. This rise was significantly and markedly modified by nicotinic acid treatment.

5.5.2 Distress, sympathoadrenomedullary activity lipid metabolism, and nicotinic acid

The exposure to experimental psychosocial stimuli caused a significant increase in the concentration of *free fatty acids* both during and after the work period in the *untreated stress group* but not in the *treated stress group* (means for 2-hour periods). The FFA increase was probably caused by an increased mobilization from adipose tissue.

According to our hypothesis (p. 91), increased activity of the sympathetic nervous system plays a significant role in the increase of FFA concentration seen after exposure to psychosocial stimuli (cf. Bogdonoff et al., 1960 b). Thus, ganglionic blockade with Arfonad® inhibits the rise

in FFA during anxiety (Bogdonoff et al., 1960 b) In the untreated stress group there was a significant, positive correlation between the changes adrenaline excretion and the changes in FFA levels between the first and the second 2 hour period ($r = 0.61$, $p < 0.05$) The corresponding correlations in the control group and in the treated stress group were, as expected, not significant ($r = 0.23$ and $r = 0.11$ respectively) The low correlation between these two variables in the treated stress group may have been caused by a selective nicotinic acid induced blockade of FFA without a corresponding influence on adrenal medullary function.

No significant correlation was found in any group between the corresponding changes in noradrenaline excretion and FFA levels. However nothing is known about the relationship if any between increased sympathetic activity *locally* in adipose tissue and the urinary excretion of catecholamines. Furthermore, the urinary catecholamines provide an index of the *mean* sympathoadrenomedullary activity during the period of urine collection, whereas the blood samples inform about the *momentary* situation as regards lipid metabolism. Finally other FFA mobilizing hormones may also have been released during and particularly after the work period and contributed to the changes in FFA level, cf. Robison et al. (1971)

The lowering effect of nicotinic acid on the concentration of FFA during the first 2 hour (resting) period is in accordance with previous results in man (Carlson and Orb, 1962 Carlson et al., 1963) The decreased (but possibly not entirely blocked, cf. figure 5 5) response of FFA to psychosocial stimuli during nicotinic acid treatment suggests an inhibition of the stressor-induced enhancement of lipid mobilization (Carlson et al., 1963) due to inhibition of lipolysis in adipose tissue (Carlson, 1963 a, Carlson, 1965).

The exposure to psychosocial stimuli was accompanied by an acute rise in the plasma *triglyceride* concentration of the *untreated stress group* without inducing concomitant changes in the *cholesterol* level, indicating an increase in the amount of triglyceride-rich but cholesterol-poor very low density lipoproteins An increase in these lipoproteins that raises the plasma triglyceride content by 0.20 mmole/liter would increase the cholesterol concentration by only about 5 mg/100 ml Such small changes in cholesterol concentration would not be detected with the technique used. Measurements of the concentration of plasma lipoproteins in medical students during stressful examination periods have shown (Grundy and Griffin, 1959 b) that the concentration of the cholesterol-rich low density lipoproteins (S_f0-12) was unaffected, while that of the triglyceride-rich very low density lipoproteins (S_f12-400) increased by 50 per cent. This is in accordance with our findings during short term distress, and with those recently reported by Taggart and Carruthers (1971) from a study of racing-car drivers.

According to our hypothesis, there is an increased mobilization of FFA from adipose tissue during distress [and probably during stress (Selye)"] eventually resulting in an elevated concentration of triglycerides in plasma. Our data support this hypothesis since the plasma triglycerides increased in the *untreated stress group* in which the concentration of FFA increased, and decreased in the *treated stress group* in which the concentration of FFA decreased. This decrease occurred before the stressor exposure and the FFA then remained at a low level throughout the study *The following rough calculations represent* an attempt to evaluate the *quantitative* aspects of our hypothesis.

5.5.3 Plasma triglycerides and the mobilization of free fatty acids: some quantitative aspects

The increase in plasma triglycerides in the untreated stress group was approximately 0.20 mmole/liter from the start of the work period (at hours) to the end of the experiment (at 6 hours) Assuming that the plasma volume was 3 liters, the mean *increase* of triglycerides in plasma works out as $3 \times 0.2 = 0.6$ mmole, which corresponds to $3 \times 0.6 = 1.8$ meq of fatty acids. Similarly the plasma pool of triglyceride fatty acids is calculated to have *decreased* by 2.2 meq in the treated stress group. If our hypothesis is

correct, the amount of FFA taken up by the liver must thus have changed by at least 2 meq in both the untreated stress group and in the treated stress group. To calculate the changes in the in flux of FFA to the liver let us assume (a) that 25 per cent of the FFA turnover is taken up by the liver (Carlson and Ekelund, 1963) and (b) that the fractional turnover rate of FFA at rest and during the work period in the untreated stress group was 0.30/minute (Fredrickson and Gordon, 1958) rising to 0.40/minute during administration of nicotinic acid when the FFA level was around 0.30 meq/liter (Carlson et al., 1963). The amount of FFA taken up by the liver at rest was thus $0.25\times0.30\times0.7\times3\times60\approx10$ meq/hour

In the untreated stress group the FFA level increased to about 0.9 meq/liter for 4 hours. The increase above the resting state of the influx of FFA to the liver was thus $(0.25\times0.30\times0.9\times3\times60\times4)-(10\times4)\approx10$ meq/liter In the treated stress group the FFA level was reduced to 0.3 meq/liter for approximately 4.5 hours. As above, it can be estimated that the hepatic uptake of FFA during this time was reduced from the basal uptake by $(4.5\times10)-(0.25\times0.40\times0.3\times3.0\times60\times4.5)\approx20$ meq The calculated changes in the amount of triglycerides in plasma, 1.8 and 2.. meq, respectively are thus much smaller than the figures for the changes in the hepatic uptake of FFA. 10 and 20 meq, respectively The changes in the hepatic uptake of FFA are thus great enough to cause changes 5—10 times greater than those seen in the plasma triglyceride pools.

Our results thus support the hypothesis but cannot establish it, as other mechanisms than those involved in our hypothesis may also have contributed to the changes observed in plasma triglyceride levels. Hepatic triglyceride output could have been stimulated in other ways than by increased flux of FFA, while during nicotinic acid treatment its output could have been reduced by other mechanisms, e g by direct interference of nicotinic acid on lipoprotein secretion in the liver Furthermore, both distress-provoking psychosocial stimuli and nicotinic acid may have influenced the fractional turnover rate of very low density lipoproteins.

5.5.4. Distress, urinary catecholamines, and nicotinic acid

According to the self ratings of our subjects, moderate distress reactions occurred during the second 2-hour period, primarily in the two groups exposed to the simulated work. Concomitantly increases in catecholamine excretion were noted, in agreement with previous findings (cf. paragraph 1.5.2)

One subject in the untreated stress group excreted 27.6, 49.7 and 21.6 ng of adrenaline per minute and 47.5 64.0 and 59.5 ng of noradrenaline per minute during the three consecutive 2 hour periods, respectively i.e. very considerable amounts, even during the two control periods. During the first 2-hour period this subject reported that he felt "calm and unconcerned" At the end of the work period, he reported that during this period it had been "rather trying but not actually unpleasant His observed behaviour however indicated relatively pronounced distress, although primarily during the work period. If for some reason, it is considered justifiable to question the "normality" of this case from the catecholamine point of view—to suppose that these high values are attributable to some intrinsic cause not relevant in this context and to exclude this subject—the two work-exposed groups differ significantly from each other in catecholamine excretion during all three periods, the excretion of the treated stress group being higher possibly due to the nicotinic acid treatment. The addition of nicotinic acid, nicotinamide, nicotinuric acid, and N-methyl-nicotinic acid in a concentration of 2 g/liter to separate urine samples did not influence the determinations of catecholamine levels performed in this study Consequently the elevated values of the nicotinic acid group cannot be ascribed to any interference from nicotinic acid or its main metabolites with the procedure for estimating catecholamines. The treated stress group exhibited a significantly higher creatinine excretion than did the untreated stress group but only during the first 2-hour period. This might have been accompanied by an increased renal clearance of catecholamines. While it is true that the effects of nicotinic acid on the sympatho-

adrenomedullary system require further study (cf the hypotheses put forward by Hoffer and Osmond, 1960) it is of major importance in our context that nicotinic acid certainly *did not inhibit* the stressor-induced rise in catecholamine excretion.

5.5.5 Cardiovascular reactions

Stressor exposure was accompanied by a brief rise in systolic and diastolic blood pressure (cf Holmberg et al. 1967) whereas rest under control conditions was accompanied by a fall. Nicotinic acid treatment did not modify the blood pressure responses. This is in accordance with previous findings that nicotinic acid does not inhibit the blood pressure response to injected noradrenaline (Carlson and Orö, 1962). Similarly our results indicate that exposure to psychosocial stimuli is accompanied by an acceleration in heart rate, which is not modified by nicotinic acid treatment. This lends some further support to our assumption that the nicotinic acid blockade of the rise in plasma lipoproteins that accompanied distress reactions is explained by an inhibition of the sympathoadrenomedullary-mediated *mobilization* of FFA from adipose tissue, and not by an inhibition of the sympathoadrenomedullary activity per se.

5.5.6 Clinical aspects

This study has clearly shown that increased plasma triglyceride levels, and probably an increased sympathoadrenomedullary activity are readily induced even by a work situation which is not real but simulated, of short duration and moderate intensity. It is tempting to speculate about the effects of the socioeconomic or other real-life stressors, which may be repeated over months and years and surely may represent a threat to the individual far exceeding that implied in our laboratory situation (Friedman et al., 1958 Levi, 1967 b and c, 1971 Raab, 1966 Cleghorn et al., 1969). The frequent finding of elevated levels of triglycerides in plasma from patients with coronary heart disease are worth considering in this context (cf. Carlson et al., 1965 a Selye, 1971 Wolf, 1971 Raab, 1971) For further discussion of these and related aspects, see Chapter 8.

5.6 Summary

Thirtythree male volunteers were studied in the morning after fasting overnight 11 (the control group) were allowed to sit comfortably for three consecutive 2 hour periods, no stressors or treatments being introduced the remaining 22 were divided into two groups, each being exposed to psychosocial stimuli (simulated work) during the second of the three 2-hour periods Following one week of premedication with nicotinic acid and after a drug-free interval of at least 16 hours, the subjects in one of these groups were each given a total dose of 3 g of nicotinic acid during the first 3 hours of the experiment, whereas the other group received no treatment.

The simulated work situation comprised monotonous but attention-demanding psychomotor performance (sorting small ball-bearings) under unfavourable environmental conditions (noise, flickering light), shortage of time and criticism. Exposure to this situation evoked moderate distress, accompanied and/or followed by increases in heart rate, blood pressure, urinary excretion of adrenaline and noradrenaline, and levels of free fatty acids and triglycerides in arterial plasma. No such increases were seen in the control group

The experimentally induced rise in free fatty acids was markedly modified by nicotinic acid treatment and triglycerides fell instead of rising, whereas the increase in catecholamine excretion was not significantly affected, neither were the increases in heart rate and blood pressure.

The hypothesis is discussed, from a qualitative as well as quantitative viewpoint, that there is a direct relationship between the increased concentration of free fatty acids accompanying distress reactions in man (and possibly evoked by an increase in sympathoadrenomedullary activity) and the eventual development of stress hyperlipoproteinemia

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6 CONDITIONS OF WORK AND SYMPATHOADRENOMEDULLARY ACTIVITY EXPERIMENTAL MANIPULATIONS IN A REAL LIFE SETTING

By Lennart Levi

6.1 The problem payment by results as an example of psychosocial stimuli in every-day life

6.1.1 Some general considerations on payment by results

One of the factors inherent in modern working life that is most often claimed to induce stress and distress in employees is payment by results, i.e. piece wages of one type or another For this reason, *and* in order to study whether or not moderate changes in psychosocial conditions of work would be effective in eliciting psychological and physiological responses, we conducted the study to be presented in this chapter

Piece work systems have in common the payment of a price or rate per piece or unit of work. This price may be uniform at all levels of output or may vary as production rises (cf. Marriott, 1957) Thus, when discussing piece-wages one must define the type referred to pure or mixed piece wages, individual or group piece wages, linear or non-linear piece wages.

Systems by which workers earnings increase more than output are based on the philosophy that the workers should benefit from the reduction of overhead costs that is achieved as output rises. Under the *high* piece rate system workers earnings are linearly related to output, as they are under *straight* piece work, but a greater increment is paid for each increase in output. For example, an increment of 1 33 per cent may be awarded to the workers time-rate for each 1 per cent increase in output.

Accelerating premium systems are based on the principle that earning increments are small for low and average levels of output, but become increasingly larger as output exceeds the average. The increments thus differ for each 1 per cent increase in output. At low output the differences are small and scarcely apparent to the worker but at high output they provide a powerful stimulus to the worker to increase his output more and more (ILO 1951)

Apart from these schemes there are bonus systems where a *major* part of the income is paid in salary form, to which is added a small bonus for each piece of work accomplished. This bonus may be linear accelerating or diminishing. The resultant incentive to work harder is comparatively slight and may even disappear at the higher performance levels.

6.1.2 Incidence of payment by results

According to statistics for the year 1969 prepared by the Swedish Employers Confederation, 63.2 per cent of the total hours worked in Swedish industry were paid by result in one form or another Similar findings have been made for workers employed by the Swedish State and local authorities (Bollinder personal communication)

In a recent nation-wide survey of wage systems in Sweden conducted by the Swedish Employers Confederation it was found that 95 per cent of the responding firms employed piece rates to a greater or lesser extent. Moreover all the large firms paid at least part of their blue-collar workers on a piece rate basis. Of the total number of hours worked, some 45 per cent were paid for by straight piece rates, while about 20 per cent were paid for by bonuses of one kind or another

The use of payment by results or bonus systems has increased somewhat in Swedish industry during the postwar period (cf table 6 1), mostly in industries where hourly rates formerly prevailed.

Table 6.1 Average volume of piece-work in Swedish industry (Source: Swedish Employers' Association).

Year	Piece-work volume in per cent of total
1950	61
1955	62
1960	64
1965	65

In Great Britain, all types of payment by results taken together cover 43 per cent of all workers in industry (33 per cent of all workers in the British economy). In the Scandinavian and East European countries and in the USSR the proportion of workers on piece-wages is higher, about 50—70 per cent of those employed by industry proper. In the USA the proportion is lower and has been estimated to some 30 per cent (McKersie, 1970).

Paying civil servants and other so-called salaried employees by unit of output is much less common. The Swedish industry with the largest proportion of salaried employees—engineering—pays less than 5 per cent of their work on this basis. The piece rate in these cases amounts to only a small bonus, the remainder of the payment being regular monthly salaries. In this sector payment by results is applied primarily in card punching routines, and, to a lesser extent, in typewriting, photoprinting, drawing and invoicing.

However, great interest has been displayed in a wider application of piece rates in white-collar employment, and—unless strong arguments are found against payment by results—we may presumably expect a fairly rapid spread during the next few years. This trend, however, is presently a matter of much controversy.

The introduction of some type of piece-wage for civil servants has been proposed by the employer in many sectors of Swedish public administration. Some of the organizations belonging to the Swedish Central Organization of Salaried Employees (TCO) have agreed to tentative experiments in this direction but have also expressed their hesitation due to occasional spontaneous negative reactions, especially from the higher age groups.

6.1.3 Payment by results: influence on productivity

Reviewing a series of laboratory experiments and field studies, Vroom (1964) concludes that a positive correlation generally exists between piece wages and productivity. The introduction of piece-wages in industry and office life has generally been found to increase productivity (Viteles, 1953; ILO, 1967; Hoffman, 1964; Richman, 1964; Edgren and Rhenman, 1970, p. 44). According to Hoffman's and Richman's descriptions, this likewise applies in the People's Republic of China and the USSR, respectively, where payment by results is said to be extensively and increasingly used.

But the co-occurrence of payment by results and high productivity does not warrant the assumption—often made by management—that the latter is necessarily a result of the former. Both may be due *to other factors, for example, more* efficient and alert management, better labour-management relations, or better organization of the work (ILO, 1967).

With reference to Swedish conditions it has been emphasized (Lindholm, 1966) that the favourable effect of piece rates on production may be explained to some degree in terms of "indirect" factors rather than the financial stimulus as such, e.g. (a) favourable attitudes of the Swedish trade unions and workers towards production advances and cost-cutting programs in general, (b) concomitant favourable effects of the introduction of piece-wages on work planning, (c) favourable effects because piece-wages have constituted a vital impelling force for the introduction of work studies, and (d) the general belief that piece-work should always entail a higher work tempo.

A search for published evidence on the effects of incentive payment systems in the form of *controlled experiments* is not very rewarding numerically, as a glance at any textbook which includes this subject will reveal. However, Marriott (1957) reviews two experimental studies worth mentioning in the present context.

Burnett (1925) conducted a laboratory experiment in order to compare the effects of time-rate

and piece rate remuneration. Four girls, aged 17 were given a highly repetitive task, two months on time rate and five weeks on piece-rate, separated by an interval of six months. On time rate they worked six hours a day for four consecutive days a week at a rate of 30 shillings a week. Attempts were made to simulate actual factory conditions. Unfortunately the author gives no details concerning the piece rate payment. The report states that during the five consecutive weeks of piece work, output increased over the average for the time-rate period by 7.2, 18.0, 20.2, 10.8 and 7.9 per cent, respectively.

Wyatt (1934) studied 10 girls in a factory under reasonably well controlled conditions. The girls, aged 15—16, worked in pairs on five operations of unwrapping, wrapping, packing, weighing and combined weighing and wrapping of chocolate and toffee. For nine weeks, each worker received a fixed time rate irrespective of output, after this, a competitive bonus system was applied for the next 15 weeks and a flat piece-rate for the following 12 weeks. It was found that both piece wage models were accompanied by substantial increases in work output. These increases came rapidly and remained relatively steady till the end of the period. A return to the original time rate for the unwrapping operation was accompanied by a substantial decrease in output. During the next nine weeks the subjects were given bonus-rates for an operation very similar to the packing process mentioned above. A considerable and progressive increase in work output occurred as compared with time-rate conditions.

These two studies and similar ones are difficult to evaluate for many reasons. The usual managerial and supervisory controls were absent. The experimental conditions introduced by the experimenters involved changes not only to the remuneration system but to many other factors as well. The groups were small and the age range was restricted to adolescents, whose financial circumstances presumably induced them to earn all the money they could.

The over-all evidence, part of which is reviewed by Locke, Bryan and Kendall (1968), nevertheless suggests that monetary incentives enhance work output. These authors also present five studies of their own supporting their hypothesis that these incentives affect task performance only through or by means of their effects on the individual's goals or intentions.

Reviewing some of the beneficial results usually expected by management, Shimmin (1959) mentions not only (a) increased output, (b) higher earnings and (c) better production control and planning, but also (d) greater cooperative efforts by workers and (e) more satisfied employees.

Briefly then, it is generally agreed that piece-wages constitute one of the most important incentives to boost productivity. It is often claimed that piece wages are a necessary prerequisite of good performance, yielding higher earnings for workers and lower costs for management.

6.1.4 Payment by results: psychological and physiological effects

In spite of this nationwide or even worldwide acceptance, little is known about the *psychological* and *physiological effects* of this remuneration system. The evidence mentioned above suggests that money strengthens motivation at work, thereby probably increasing the intensity and endurance of employees' performance, but at the same time it is conceivable that excessively strong motivation, if prolonged, could lead to undue strain on psychological and physiological mechanisms. The urge for or need of money may temporarily or permanently seduce the individual to ignore psychological and physiological warning signals such as physical and mental fatigue, nervous tension, dysfunction of organs and organ systems etc.

Yoder (1947) has pointed out that piece-wages may encourage a disregard for essential health considerations, because "workmen, when they are liberally paid by the piece, are very apt to overwork themselves, and to ruin their health and constitution in a few years".

Discussing possible disadvantages inherent in piece-work, Marriott (1957), too, mentions among other things

(a) a tendency for quality to deteriorate

(b) a danger of disregarding safety regulations and thereby increasing accidents
(c) a tendency by some workers to overwork and to undermine their health
(d) jealousy among workers because some are able to earn more than others.

Guttormsson and Smith (1971) report a study of attitudes among 4,705 subjects in Swedish civil administration (National Postal Bank). A majority of the subjects were women. All were working at departments where piece-wages had already been introduced or would be within the next year or two. Out of those who were on salary 45 per cent expressed positive attitudes toward a mixed piece wage system, compared with 39 per cent of those already on piece-wages. The corresponding negative attitudes were expressed by 37 per cent and 42 per cent, respectively. Attitudes were generally more negative among the over 40s. Subjects working under *individual* piece-wage plans were more positive towards piece-wages in general than those working on group piece-wages. 51 per cent of those on individual and 25 per cent of those on group piece-wages described a decreased propensity to help fellow workers since the introduction of piece wages. 47 and 57 per cent respectively reported no change in this respect. Out of those on salary 73 per cent believed that piece-wages would lead to a loss of comfort and well-being. Out of those working on individual and group piece-wages, 60 per cent and 70 per cent, respectively, reported a decrease in comfort and well-being since the introduction of piece-wages. A great majority of all subjects reported that they envisaged, or had experienced, an increased feeling of distress in response to piece wages.

This aspect—the relationship between piece wages and reported experience of distress—has been studied in more detail in two sociological investigations. Gardell (1971) found that industrial workers who were paid by the piece (in combination with low income and unskilled tasks) rated lower on a number of mental health variables. Similarly, Ohlström (1970) and Bolinder and Ohlström (1971) identified piece-wages as a factor contributing to subjective feelings of dissatisfaction and distress, respectively. However none of these investigations aimed at quantifying psychological and physiological *reactions* to the piece-work situation, only measuring attitudes to piece wages.

6.2 Choice of methodology

The above constituted the background for the study to be reported in this chapter. If reliable data could be produced on the stress and distress evoked by different work routines and modes of management in various professions, this would have obvious implications for activities such as the engagement of new employees, transfers within a given company, vocational guidance, safety precautions, and industrial hygiene, and also with regard to the fair assessment of remuneration, mental hygienic evaluation of proposals for new processes, streamlining and automation.

It is also important clinically to know how the human organism adapts or fails to adapt to certain minor but often repeated, psychosocial stimuli of an *every-day* character.

In order to furnish at least part of the basis necessary for such developments, we have exposed groups of subjects to conditions of work that are often assumed to constitute significant stressors in modern working-life.

These conditions included those facing telephone operators, invoicing clerks working on a salary and piece-wage basis, office clerks subjected to changes in work environment (conventional offices, office landscapes, different noise levels), supermarket cash desk girls (during rush hours and ordinary conditions), supervisors in engineering, and paper mill workers and engine drivers working in various shifts.

The responses to these exposures were assessed with respect to psychological self-ratings by the subjects, performance was evaluated, subjectively and—wherever possible—objectively, and sympathoadrenomedullary activity was measured as the urinary excretion of adrenaline and noradrenaline.

The great majority of experiments in the field of psychophysiological research have utilized

and piece rate remuneration. Four girls, aged 17 were given a highly repetitive task, two months on time rate and five weeks on piece rate, separated by an interval of six months. On time rate they worked six hours a day for four consecutive days a week at a rate of 30 shillings a week. Attempts were made to simulate actual factory conditions. Unfortunately the author gives no details concerning the piece rate payment. The report states that during the five consecutive weeks of piece work, output increased over the average for the time rate period by 7.—, 18.0, —0.2, 10.8 and 7.9 per cent, respectively.

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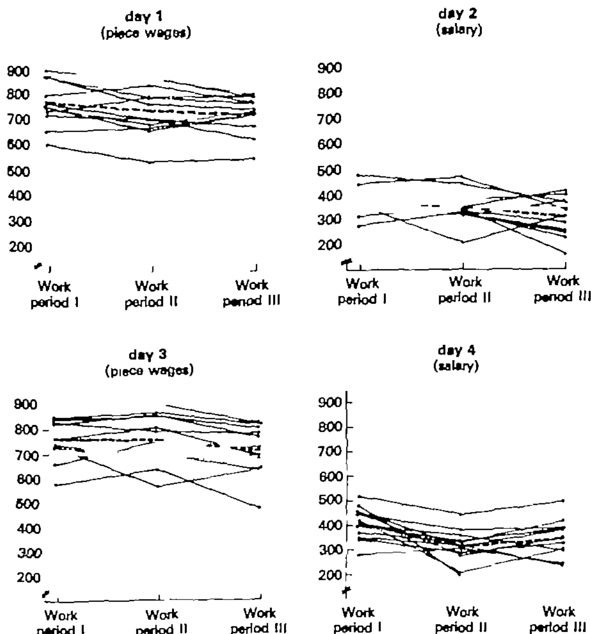


Figure 6.2 Individual performance during each of the three periods of work under piece-wages (days 1 and 3, left) and salaried conditions (days 2 and 4, right). Dashed line indicates means.

task, in their usual environment and under their usual supervisor who knew them well. The task involved performing on an invoicing machine a few simple mathematical operations, the result of which was then typed on a postal paying-in form and on a check slip.

On the *second* and *fourth* of the four experimental days, remuneration was on the customary basis, namely the modest monthly salary. On the *first* and *third* days, on the other hand, a system of piece-wages was introduced. As the most extreme form of piece-wages was considered to be

the non-linear progressive, individual model, a piece wage of this type was added to the ordinary salary if and when the subjects performance exceeded 160 invoices per hour (figure 6 1) This level was chosen as a suitable base-line because previous work studies, performed by the Administration a few months earlier without the employees knowledge, had demonstrated in this group of invoicing-clerks a habitual mean work output of approximately 160 invoices per head and hour of work (range 122—195)

The system included a moderate deduction for each subject s miscalculations and typing errors, by eight invoices per hour for each per mille of errors (habitual mean 2.5 per mille, range 0.0—6.8 per mille).

The number of invoices per time unit, and the mean per mille of invoicing errors were established by a cross-check made by the experimenter s clerical assistants.

Except for the change in remuneration, the experimental setting was held constant. The individual work output and the number of errors were kept secret from the employer as well as from fellow workers in order as far as possible, to eliminate pressures from these sources, be they real or imagined, on the individual invoicing-clerk.

Subjects e reactions (rushed, tired, physical discomfort—in Swedish jäktad, trött, kroppsliga obehag) were assessed on simple 4-point rating scales ranging from "very (rushed etc.) = 4 points, over fairly = 3 points, and slightly = po nts, to not at all" = 1 point (in Swedish mycket, ganska, något, inte alls).

Briefly then, a strong monetary incentive was introduced on days 1 and 3 but only for a work output exceeding the habitual level. This system was chosen in order to mimic qualitatively (although not quantitatively) what industrial management actually might do to eliminate a bottleneck in a production process. Thus, the study was designed as a factorial experiment with the factors (a) salary versus piece w ges, (b) first ersus second day of presentation of each mode of remuneration, and (c) morning ersus fternoon Five differences of special interest were calculated

Table 6 2. Means and S.E.M. for output of work (Invoices per 2 1/2 hour period, each comprising 2 1/4 effective working-hours, during days 1 and 3 (piece wages) and days 2 and 4 (salary)).

Day No.	Condition	Period	Work output (Invoices) Mean ±	S.E.M.
1	Piece-wages	A	771.8	26.4
		B	736.9	28.2
		C	719.8	21 7
2	Monthly salary	A	364 4	15 4
		B	343 7	18 6
		C	308 6	21.8
3	Piece-wages	A	765.2	27 0
		B	762.1	30 4
		C	711.0	29 0
4	Monthly salary	A	409 3	19.3
		B	314.5	19 6
		C	347 4	21.3

A stands for the period 0—2 1/2 hours, *B* for 2 1/2—5 hours, and *C* for 5—7 1/2 hours.

and will be reported below namely between (a) piece wages and salary (b) afternoon and morning hours, (c) afternoon and morning hours during piece-wages and salary (d) second and first pair of days, and (e) piece wages and salary on second and first presentation, table 6 6.

6 4 Results

6.4.1 Differences in reactions to salary and piece-wages

6 4.1.1 Output

During the two days on salary only the subjects performed at a mean rate of 155 invoices per hour and head, i.e. very close to the predetermined habitual level of 160. During the two days with piece wages, output more than doubled, to 331 invoices per hour and head ($p < 0.001$) cf figure 6 and table 6.

In spite of this very considerable increase in output, the number of errors remained very low (mean 3.0 per mille, range 0.6—8.1 per mille), not significantly different from the habitual level reported above

Table 6.3. Means and S.E.M. for self-ratings (rush, fatigue, physical discomfort) for each period during days 1 and 3 (piece-wages) and days 2 and 4 (salary).

			Rush		Fatigue		Physical discomfort	
Day No.	Condition	Period[a]	Mean ±	S.E.M	Mean ±	S.E.M	Mean ±	S.E.M
1	Piece-wages	A	1.75	0.25	1.50	0.20	1.33	0.19
		B	1.50	0.20	2.17	0.27	1.9	0.‿6
		C	1.83	0.27	2.58	0.34	2.17	0.35
2	Monthly salary	A	1.00	0.00	1.25	0.25	1.00	0.00
		B	1.00	0.00	1.25	0.13	1.00	0.00
		C	1.08	0.08	1.33	0.14	1.00	0.00
3	Piece-wages	A	1.08	0.08	1.33	0.14	1.33	0.19
		B	1.4‿	0.19	2.33	0.31	1.75	0.31
		C	1.83	0.30	2.92	0.23	2.25	0.41
4	Monthly salary	A	1.00	0.00	1.33	0.14	1.25	0.25
		B	1.00	0.00	1.67	0.26	1.25	0.25
		C	1.00	0.00	1.75	0.22	1.42	0.26

The ratings were made on 4-point scales ranging between "much" (4 points) and "not at all" (1 point).
[a] *A* stands for the period 0–2 1 2 hours, *B* for 2 1 2–5 hours, and *C* for 5–7 1 2 hours.

6.4.1.2 Subjective reactions

During salaried days, the "rush" and "physical discomfort" scores were all on a very low level only a few individual girls occasionally reporting sensations of this type. Under piece-wage conditions these rating scores rose significantly ($p < 0.01$) but the *mean* levels were still below or around 2 points (i.e. "slight" feelings of rush and physical discomfort) cf table 6 3

Fatigue ratings also increased significantly ($p < 0.01$) from salaried to piece wage conditions, reaching "moderate" (= 3 points) mean levels towards the end of the piece work days, cf table 6 3

On the morning of the forth day (salaried work) one of our subjects started to complain about pronounced menstrual pain. After having completed the first two work periods of that day

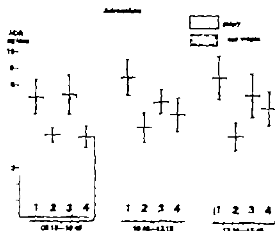


Figure 6.3 Means and S.E.M. for adrenaline excretion during each period of work during piece-work conditions (days 1 and 3 filled bars) and on salaried days (days 2 and 4, white bars).

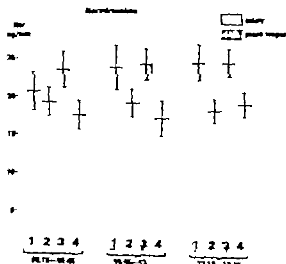


Figure 6.4 Means and S.E.M. for noradrenaline excretion during each period of work during piece-work conditions (days 1 and 3, filled bars) and on days (days 2 and 4 white bars).

Table 6.4 Means and S.E.M. for urinary excretion of adrenaline and noradrenaline for each period during days 1 and 3 (piece-wages) and days [illegible] and 4 (salary).

Day No.	Condition	Period[a]	Adrenaline ng/min Mean ±	Adrenaline ng/min S.E.M.	Noradrenaline ng/min Mean ±	Noradrenaline ng/min S.E.M.
1	Piece-wages	A	7.33	1.03	20.62	2.90
		B	8.52	1.13	23.81	2.89
		C	8.44	1.28	[illegible]4.46	[illegible].33
	Monthly salary	A	5.0[illegible]	0.46	19.20	1.86
		B	5.46	0.88	19.16	1.77
		C	4.97	0.82	18.22	1.47
3	Piece-wages	A	7.49	1.21	23.49	2.36
		B	7.00	0.72	24.33	1.98
		C	7.38	1.27	[illegible]4.49	1.80
4	Monthly salary	A	4.87	0.66	17.55	1.91
		B	6.20	1.01	17.16	3.31
		C	6.98	1.00	19.11	1.55

A stands for the period 0–1 1/2 hours, *B* for 1 1/2–5 hours, and *C* for 5–7 1/2 hours.

she left for home. Her adrenaline excretion levels during these two periods were about twice as high as those from the corresponding periods of day

6.4.1.3 *Physiological reactions*

There was a highly significant increase in adrenaline as well as noradrenaline excretion ($p < 0.001$) under piece-wage conditions, cf. figures 6.3 and 6.4 and table 6.4. Significant changes ($p < 0.001$) were also found for the increase in urine flow and decrease in specific gravity (*figures 6.5 and 6.6*, and table 6.5). Creatinine excretion, too, increased ($p < 0.05$) during the days on piece-wages, cf. table 6.5.

6.4.2 Differences in *reactions during morning and afternoon hours*

6.4.2.1 *Output*

Throughout the study output decreased significantly ($p < 0.001$) *from the first working period* (8.30–10.45 a.m.) *to the third* (1.30–3.45 p.m.), cf. *table 6.6*.

Table 6.5 Means and S.E.M. for urinary creatinine, urine flow and specific gravity for each period during days 1 and 3 (piece-wages) and days [illegible] and 4 (salary).

Day No.	Condition	Period	Urinary creatinine mg/min Mean ±	Urinary creatinine mg/min S.E.M.	Urine flow ml/min Mean ±	Urine flow ml/min S.E.M.	Specific gravity (N−1)×1000 Mean ±	Specific gravity (N−1)×1000 S.E.M.
1	Piece-wages	A	1.11	0.18	2.[illegible]4	0.17	8.03	0.69
		B	0.80	0.04	1.60	0.16	9.42	1.10
		C	0.68	0.04	1.46	0.12	9.[illegible]5	0.99
	Monthly salary	A	0.83	0.03	1.[illegible]8	0.1[illegible]	11.83	1.[illegible]5
		B	0.75	0.10	1.06	0.11	13.00	0.97
		C	0.65	0.05	1.21	0.10	13.08	0.89
3	Piece-wages	A	0.83	0.04	1.73	0.[illegible]1	11.33	1.65
		B	0.73	0.03	1.76	0.14	9.33	1.03
		C	0.65	0.04	1.6[illegible]	0.10	9.17	0.55
4	Monthly salary	A	0.75	0.05	1.57	0.17	1[illegible].50	1.39
		B	0.77	0.05	1.31	0.13	12.00	1.76
		C	0.75	0.03	1.61	0.1	9.33	0.89

A stands for the period 0–1 1/2 hours, *B* for 1 [illegible]–5 hours, and *C* for 5–7 1/2 hours.

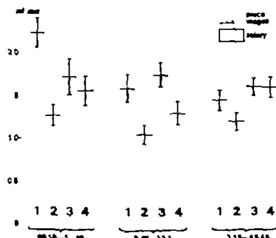


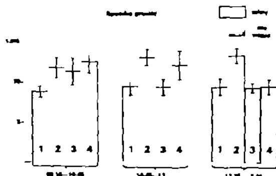
Figure 6.5 Means and S.E.M. for urine volume during each period of work during piece-work conditions (days 1 and 3, filled bars) and on salaried days (days 2 and 4 white bars).

6 4.2.2 Subjective reactions

"Fatigue scores increased significantly ($p < 0.001$), as did those for "physical discomfort" ($p < 0.05$), whereas the changes in "rush" scores did not reach significance, cf table 6 6.

6 4.2.3 Physiological reactions

Neither adrenaline nor noradrenaline excretion rates exhibited any significant changes from morning to afternoon hours, whereas urine volume decreased ($p < 0.05$) as did creatinine excretion ($p < 0.01$). No significant changes were found in specific gravity cf. table 6 6



Figur 6.6. Means and S.E.M. for urinary specific gravity during each period of work during piece-work conditions (days 1 and 3 filled bars) and on salaried days (days 2 and 4 white bars)

6.4.3 Differences between morning and afternoon hours under salaried and piece-work conditions

The increases in "fatigue and "physical discomfort" ratings were significantly higher during piece-work than during salaried work, ($p < 0.01$ and $p < 0.05$ respectively) as was the decrease in urine volume ($p < 0.01$) For the other variables studied, no significant differences were found, cf. table 6 6.

6.4.4 Differences in reactions during first and second pair of days

No significant changes occurred from the first two days to the last two in any of the variables under study cf table 6 6.

6.4.5 Differences between the two remuneration models on first and second presentation

The difference between the two remuneration conditions turned out to to be significantly greater on the first than on the second presentation day for urine volume ($p < 0.05$) specific gravity ($p < 0.05$) and creatinine ($p < 0.01$) but not for any of the other variables, cf. table 6 6.

6 5 Discussion

6.5.1 Differences between salary and piece wages

Every psychological and physiological variable covered by this study turned out to be influenced by the change in remuneration system. As our subjects were free to choose their performance rate under both conditions of work, it must be concluded that the piece-wage incentive reinforced the motivation to work at a higher rate. It is, however extremely unlikely that the subjects would have been able to maintain their output at the very high level found during the piece-work days for any length of time.

It is often claimed (Shimmin, 1959) that such an increase in productivity is accompanied by a corresponding decrease in the quality of work. This was, however not the case to any marked degree under the conditions of our study Again, it is *conceivable* that a long term experiment would have given different results.

Table 6·6 Means, S.E.M. and tests of significance for work output, subjective reactions, and physiological reactions with respect to differences between (a) piece-wages and salary (b) afternoon and morning hours, (c) afternoon and morning hours during piece wages and salary (d) second and first pair of days, and (e) piece wages and salary on first and second presentation.

	DIFFERENCES BETWEEN									
	piece-wages and salary		afternoon and morning hours		afternoon and morning hours during piece-work and salaried conditions		second and first pair of days		the two remuneration models on second and first presentation	
Variable	Mean ±	S.E.M	Mean ±	S.E.M	Mean ±	S.E.M.	Mean ±	S.E.M.	Mean ±	S.E.M.
Work output (invoices)	396.35	25 62	−56.15	9.90	5 38	16.62	10.85	8.53	−14.64	14.95
Rush	0.56	0 15	0.23	0.14	0.38	0.31	−0.14	0.09	−0.22	0 16
Fatigue	0 71	0 16	0.79	0 14	1.08	0.25	0.21	0 12	−0.19	0.22
Physical discomfort	0 64	0.19	0.48	0 17	0.79*	0.29	0.14	0.18	−0.33	0.19
Adrenaline ng min	2.11	0.45	0.77	0.72	−0.53	0.87	0 03	0.45	−1 68	0.80
Noradrenaline ng min	5 13	0.86	1.35	0.68	2.13	1.81	0.11	1.22	2.06	1 70
Urine volume ml min	0 40*	0.08	−0.23	0.09	−0.43	0 12	0.13	0.08	−0.38	0.17
Specific gravity (N−1)×1000	−2.53	0 49	−0.73	0.79	0.46	1.20	−0.17	0.59	2.39	0.95
Urinary creatinine mg min	0.05	0.02	−0.20*	0.05	−0.22	0.12	−0.06	0.04	−0.15	0.05

This may also apply to "rush" "physical discomfort" and "fatigue" ratings, all of which increased significantly during piece work days. Nothing definite can be concluded about whether or not the increase in these ratings was due to the piece wages per se, or to the increase in output. Although the *means* for these ratings never reached more than "slight" or "moderate" intensity the questionnaire returns as well as clinical observations support the assumption that individual girls worked at a supraoptimal rate and experienced relatively pronounced discomfort.

Catecholamine and creatinine excretion, urine flow and specific gravity were all significantly affected by the remuneration system. Mean adrenaline excretion was about 40 per cent higher during piece work compared with salaried work.

It might be argued that this increase in adrenaline excretion reflects a corresponding increase in muscular work. But although muscular work has been demonstrated to affect adrenaline excretion, this is only true of rather high levels of exertion (Frankenhaeuser et al 1969) Therefore, it seems more justified to assume that this increase primarily reflects a corresponding increase in distress. This also applies to the other physiological reactions, these being of the same direction and order of magnitude as those described during short-term exposure to other types of psychosocial stimuli. In the case of noradrenaline and creatinine excretion, however the effect may have been mixed, comprising also the consequences of increased muscular activity

6.5.2 Differences between morning and afternoon hours

The changes in variables over hours of work are relatively small and represent a combined effect of circadian variation and the duration of work. The increases in "fatigue" and "physical discomfort" ratings and the decrease in output are in

accordance with every-day experience. The changes in urine volume and creatinine excretion *are probably due to the circadian variation demonstrated to occur during these hours of the day* cf. paragraph 2.7

6.5.3 Differences between morning and afternoon hours during *salaried versus piece-work* conditions

Our findings support the assumption that the remuneration system, and not just circadian variation, is responsible for some of the reactions occurring from morning to afternoon hours.

6.5.4 Differences between first and second pair of days

Several other investigators have found that an increased familiarity with the experimental situation results in decreases in psychological and physiological reactions to it. Thanks to the pre experimental dress-rehearsal day" included in our study all subjects were to some degree accustomed to their routine and the experimental procedure. One might hypothesize this to be the reason for no significant effects occurring from a repetition of the exposure, though of course it is conceivable that the absence of any significant changes was due to a combined effect of habituation (decreasing the levels) and prolonged exposure (increasing the levels), resulting in no net change.

6.5.5 Differences between the two remuneration models, first and second presentation

The results speak in favour of a diminishing difference between the reactions to the two remuneration models when exposure to them is repeated. W do not know whether this difference would diminish still more after prolonged exposure or even disappear altogether Probably this would depend on the interplay between the characteristics of the piece-wage system and the expectations, needs, attitudes and other characteristics of the individuals.

Clearly the object of the present study was not to describe the psychosomatic health hazards, if any or the relative efficiency of the remuneration systems. The aim was simply to compare the effects of two every-day settings with respect to *their effects on psychological, behavioural and* physiological variables. The results support the assumption that psychosocial factors of an every day type have, indeed, significant effects on the functions under study It was further found that a real-life setting can be used in the study of psychosocial influences on psychophysiological reactions.

6 6 Summary

Following a dress rehearsal day 12 healthy female invoicing clerks were studied under conditions very similar to those involved in their every-day work, a number of extraneous physical and psychosocial stimuli, however being kept under control. Highly progressive piece-wages were introduced on the first and third day of the experiment, and were found to result in significant increases in output but also in rush, fatigue and physical discomfort ratings, in adrenaline, noradrenaline and creatinine excretion and in urine flow with a concomitant decrease in specific gravity The implications, clinical and methodological, of these results are discussed.

6 7 Acknowledgements

This investigation was made possible by the kind cooperation of Mr Harry Westerberg, Director and his colleagues of the Swedish Telecommunications Administration, which is gratefully acknowledged. The Swedish Central Organization of Salaried Employees, the Swedish Confederation of Trade Unions (Dr Erik Bolinder), the Swedish Employers Confederation (Dr Nils Masreliez), and Drs. Ake Swensson and Bertil Gardell have all furnished valuable advice and background information for this study for which the author *expresses his gratitude. The* original study was supported by a grant from the Swedish Union of Clerical and Technical Employees in Industry The final compilation of the data was supported by a grant from the Bank of Sweden Tercentenary Fund.

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7 PSYCHOLOGICAL AND PHYSIOLOGICAL REACTIONS TO AND PSYCHOMOTOR PERFORMANCE DURING PROLONGED AND COMPLEX STRESSOR EXPOSURE

By Lennart Levi

7.1 The problem

All studies reported in the previous chapters have been of relatively short duration, partly because such studies are easier to conduct and partly because they were focused intentionally on rather acute reactions to short-term stimuli. Whereas we know that catecholamine excretion in man changes rather rapidly when the organism is exposed to psychosocial influences, other clinically relevant physiological and biochemical variables may necessitate stimulation—or at least a time lag—of considerably longer duration before reactions materialize. Here, then, was the main reason for making repeated measurements over a rather long period, comprising at least several days. An additional reason was that it is usually assumed that the psychosocial stimuli of real life which really are of pathogenic significance, are relatively long lasting. At least they certainly usually do act on the organism for considerably longer than just an hour or two or even a day. It therefore seemed to be of considerable interest to study the psychological and physiological reactions of the human organism to relatively *prolonged* exposure.

True, a number of studies have been published which involved stimuli that lasted several days, but many studies of this type have either been conducted under poorly controlled conditions, the subjects often being allowed to smoke, drink coffe and choose the work/rest cycles they preferred, or the measurements have comprised either psychological or physiological or performance variables, but usually not a combination of these.

In view of the above, the present study was designed to include the following objectives.

First, we intended to study changes over time in self-ratings of distress" and fatigue under strictly controlled environmental conditions.

Second we intended to collect data on changes in *catecholamine excretion* in response to prolonged exposure to presumably distress-provoking stimuli under controlled conditions. Would the increased excretion elicited by short term exposure persist at the enhanced level, continue to increase, or be brought back to—or even below—the control level by some homeostatic mechanism?

Third, although it was known that catecholamine excretion was higher during waking than during sleeping hours, available data did not clearly indicate whether this difference was due to rather acute effects of Zeitgebers such as (a) participation in physical and mental activities, (b) bodily posture, (c) eating and drinking, (d) smoking and (e) a generally higher sensory input, as opposed to some intrinsic (circadian) rhythms.

Fourth, as mentioned in paragraph 1.5.4 existing information was rather inconclusive as to the influences of psychosocial stimuli on the release of *thyroid* hormones in man. In view of our ignorance concerning the etiology and pathogenesis of Graves disease, and the pronounced influence of these hormones on a great number of physiological and biochemical variables in health and disease, inter alia interacting with the catecholamines, it seemed important to study protein-bound iodine (PBI) as an index of thyroid function under the influence of a rather prolonged stressor exposure.

Fifth, as emphasized by Malmström (1970) and many others, *iron* plays a central role in the

energy metabolism of all living cells. There exists a labile iron exchange compartment which is in equilibrium with the plasma stores (for a review see Najean et al., 1970) and changes in plasma iron levels are accordingly bound to reflect some of the changes in iron kinetics. Plasma iron is known to exhibit a circadian rhythm (Vahlquist, 1941 Hemmeler 1944 Høyer 1944 Waldenström, 1946 Hamilton et al., 1950 Laurell 1953 Perkoff et al., 1959 Speck, 1968) It is further reported that serum iron levels decrease in response to infections (for a review see Bothwell and Finch, 1962) and 2—3 days before the onset of menstruation (cf. Zilva and Patston, 1966) to gross cerebral stimuli such as short wave irradiation of the brain-stem, lumbar puncture, pneumoencephalography and intraventricular bleeding (Laurell, 1952 Schäfer 1964) and to injections of histamine, adrenaline, adrenal cortical extracts and ACTH (for a review see Laurell, 1957) Moreover Liljedahl et al. (1969) have demonstrated an accelerated elimination of plasma iron following meniscus or hernia operations with minimal blood loss. Phlebotomy of comparable volume to the loss by bleeding and haemolysis (100—150 ml) did *not* accelerate iron elimination.

Low serum iron levels have been reported in schizophrenic patients (Frohman et al., 1958) and in psychiatric patients shortly after admission to a mental hospital (Skang, 1970 Amdisen, personal communication) and iron deficiency is often accompanied by mental fatigue, lack of drive, lack of concentration, loss of energy and rapid mental exhaustion (Heilmeyer and Harwerth, 1970)

Briefly then, serum iron levels seem to be influenced in a rather stereotyped way by a considerable number of diverse stimuli that possibly act through a common neuroendocrine pathway conceivably activating the reticuloendothelial system (Schäfer and Boenecke, 1949 Laurell 1952 Lederer 1962 Zilva and Patston, 1966) and iron metabolism, in turn, exercises a profound influence on a great number of organs and organ systems. Accordingly one of the aims of the present study was to investigate whether changes in serum iron could be induced by experimental stress exposure of young, healthy male subjects receiving an adequate supply of alimentary iron.

The study also comprised several other physiological and biochemical variables that will not be reported in detail in the present context. As some of these data may help to elucidate the problems under discussion, they will be mentioned, but only in the discussion.

7.2 Choice of methodology

In order to achieve the objectives indicated above, we needed an experimental situation that could be expected to induce distress (and stress) reactions of a prolonged nature but not so pronounced as to be harmful to the population in question. The situation should further be rather homogeneous with respect to the stimuli applied (to allow systematic studies of changes in reactions over time as well as the detection of circadian rhythms) and should allow simultaneous assessments of performance (as to quality and quantity), subjective reactions, and the physiological and chemical variables mentioned above.

To accomplish this, we designed an electronic shooting range and created a situation in it that our subjects, who were officers and soldiers, would presumably recognize as involving some of the elements of prolonged ground combat. This "pseudorealistic" setting was rather convenient for the assessment of psychomotor performance (quantity and quality) and it also kept the subjects uniformly occupied. Furthermore, the task, which was of a vigilance type, was considered rather meaningful for military subjects.

Briefly then, the procedure was assumed to be meaningful to the subjects, kept them uniformly occupied throughout the study did not allow self-chosen periods of rest, allowed studies focused on stress, distress and circadian rhythms, made possible the collection of urine and blood samples at predetermined intervals and generally made it possible to study psychomotor performance, subjective reactions and physiological and biochemical reactions under strictly controlled environmental conditions.

7.3 *Material, methods and procedures*

7.3.1 The subjects

The 31 subjects of the study were Army officers and corporals attending platoon-leader training school, with an age range of 20 to 44 years, the mean being 29.

They were in excellent health and either non-smokers or able to give up smoking for the days of the experiment. All the subjects volunteered for this study after they had received precise written information about its aim and the procedure involved (informed consent).

The Army officers were selected from a large sample of regular and reserve officers who had responded to a memorandum inviting them to participate. To qualify every subject had to pass a thorough medical examination, including ECG at work. Only those who were found to be in perfect health were allowed to participate. The corporals were recruited in a similar way some probably being motivated by professional interest, others by a desire for a new thrill or the medical check-up offered as part of the investigation, or by the monetary reward (Swedish Kronor 200 — i.e. approximately US $40 —).

Clearly this procedure resulted in an experimental group that did not form random sample of the populations from which it was drawn on the contrary the group constituted a highly selected pick. Many of the subjects were active sportsmen. As judged by their superior officers they constituted a positive selection, physically and intellectually as well as according to military criteria. However in view of the assumption that the strain on the subjects would be considerable, this selection was considered necessary on ethical grounds.

7.3.2 The procedures

7.3.2.1 Pre-experimental procedures

During the week prior to the experiment, each subject was allowed to adapt to the test situation, adjust the telescopic sight and support of his rifle, choose the most convenient height for his adjustable office-chair, and do some trial shooting. He was also carefully instructed in and informed about all the details of the experimental procedure in order to secure maximal co-operation. During this week, the subjects also underwent a comprehensive check up including (a) a medical investigation, (b) a psychiatric investigation, (c) two personality inventories, (d) a bicycle ergometer maximal work capacity test, including ECG and (e) biochemical parameters.

A pre-stress blood sample was obtained on either Wednesday or Thursday of the pre-experimental week, between 12.00 noon and 3.00 p.m. The venous puncture was always preceded by restriction on physical activity for at least four hours: this time was used to inform and instruct the group about the experimental routine, to demonstrate the premises, and to complete the questionnaires. Food and fluid (two standard sandwiches with ham, 300 ml tap water) were administered every three hours from awakening until three hours before the blood sample was collected, all tobacco, coffee and alcoholic beverages as well as all unscheduled activities being strictly prohibited during the same period. None of the subjects was or had recently been on any drug regimen whatsoever.

The experiment, run in 1965 was conducted in two shifts, four weeks apart, the first with 15 subjects, the second with 16. As the procedures and results were practically the same for both shifts, they will be reported together. Preliminary accounts have been published (Levi, 1966, 1967).

7.3.2.2 The experiment proper

The experiment started on a Tuesday morning. On awakening, the subjects, having fasted over night, emptied their bladders, drank 300 ml of tap water and reported at the laboratory as indicated below. At the beginning of the control period they again emptied their bladders, drank 300 ml of tap water and were served two standard sandwiches (with ham and beef). This procedure was repeated at 3-hourly intervals throughout the experiment, which ended on Friday a good 75 hours later. In this way each subject produced 25 urine samples from the same number of 3-hour periods.

During the 3-hour control period (for half the group 8.00 a.m.—11.00 a.m. for the other half

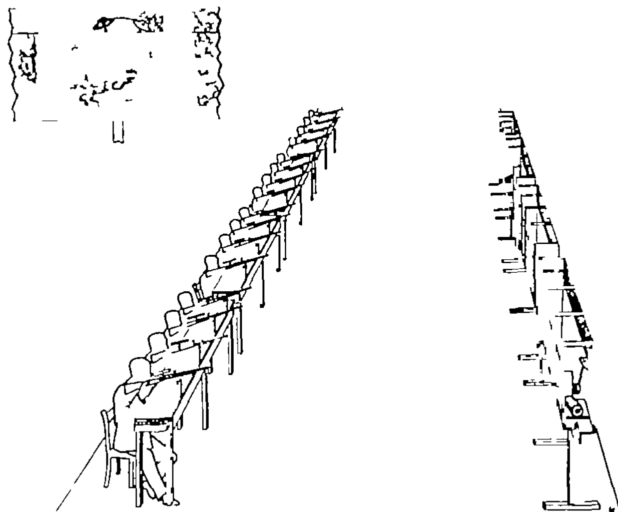


Figure 7.1 Schematic drawing of the shooting range. The target area is shown in the left pper corner

11.00 a.m.—02.00 p.m.) the subjects relaxed, listening to soft music, reading weekly magazines, or just dozing. During the subsequent 72 hours, divided into 4 3-hour periods, they were exposed to the experimental conditions, which simulated some of the elements of war

The situation involved shooting on a specially designed shooting range (figure 7 1) with elec tronic rifles (producing light beams) at small targets (tanks) containing photo-diodes. The tanks moved across the field of vision at unpredictably varying speeds, disappeared behind the horizon of the shooting-range and reappeared again after a perpetually changing interval Each shot and each hit was registered electronically and individual results were noted at the end of each 3-hour period. Shortly after the end of each period, the individual number of shots and hits obtained during the period was reported to each subject in order to increase his motivation. The optimal shooting rate was one shot per second during the time the target was visible over the horizon. This rate was indicated by small flashing lights in the neighbourhood of the target area. The entire target area was operated automatically

In alternate periods the pendant lamps were turned on, which made the shooting task relatively easy In the intervening periods, however the lights were off, the only source of illumination now being weak footlights near the target area.

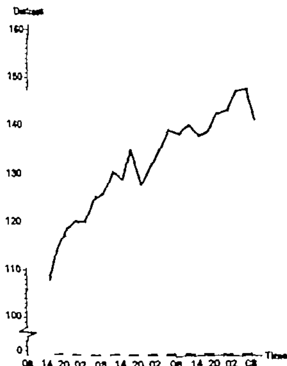


Figure 7.2. Means ± S.E.M. of self-rated distress (magnitude estimations). Control level by definition = 100 for control period 08—11 and 11—14 hours, respectively. Black markings on time scale indicate periods with bad illumination and high levels of battle noise.

In addition, in these periods an authentic battle noise from a tape-recorder was played, amplified to a level of approximately 95 dB-C.

An unabated 2 3/4 hours of such activity was followed by a concentrated 15-minute period for answering questionnaires, ingestion of the standard meal described above, voiding urine for analysis, and attendance to other toilet functions. After this pause, the shooting range was switched on again, and in this manner the experiment was continued for three days and nights.

Throughout the experiment, members of the research staff prevented the subjects from falling asleep or turning away from their task. If some one fell asleep, he was roused immediately and told to go on shooting. The subjects were continuously supervised by the experimenters and their assistants, one being in the shooting-range, the other watching via closed-circuit television in an adjoining room, communicating with the subjects through the amplifiers.

The physical setting was such that the activity was performed in a large room, isolated from the rest of the hospital. There were no windows nor any other means of communicating with the outer world, and the subjects were deprived of their watches in order to minimize any cues as to time of day. No activity but the experimental one was allowed. The subjects had to sit on their chairs all the time except when voiding.

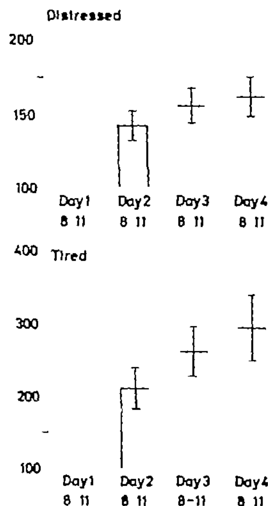
A "post-stress" blood sample was obtained after at least 72 hours of stimulus exposure, in each subject at the same time of day as the "pre-stress" sample ±30 minutes, being preceded by the same food and fluid at the same interval as mentioned above.

7.3.3 Methods of measurement

Self-ratings of distress and fatigue were made every 3 hours by the magnitude estimation method (cf. paragraph 2.18) and on 11-point rating scales (cf. paragraph 2...0 7.2). By definition, the amount of "distress" and "fatigue" at the beginning of the vigil was given the value of 100 in the magnitude estimations. During each of the subsequent 3-hour periods, the subjects were asked to report their average 3-hour "distress" and "fatigue" in per cent of this initial level.

Performance in the psychomotor task on the shooting range was evaluated as to speed and accuracy simply by reading off the individual counters for shots and hits.

Serum iron was analyzed according to Agner (1955) and protein-bound iodine by the method of Riley and Gochman (1964). The samples from the different days for each subject were always analyzed in a single sequence and by the same laboratory technician. The standard deviation in blind analyses of duplicate samples within the range of values found by us has been investigated previously and amounted to 4.9 μg/100 ml plasma for serum iron (Strandberg, 1966) and 0.3 μg/100 ml plasma for protein-bound iodine (Crowley and Jensen, 1965).



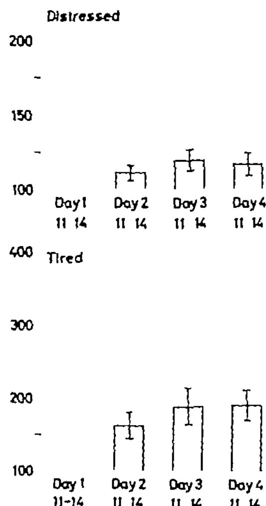


Figure 7.3 Self-rated distress and "fatigue" (magnitude estimations) during the control period (day 1 08—11 hours for half the group 11—14 hours for the other half) as compared with the corresponding periods of days 2, 3 and 4. Means ± S.E.M

7 4 Results

7.4.1 Behaviour and performance

7 4 1.1 Self ratings

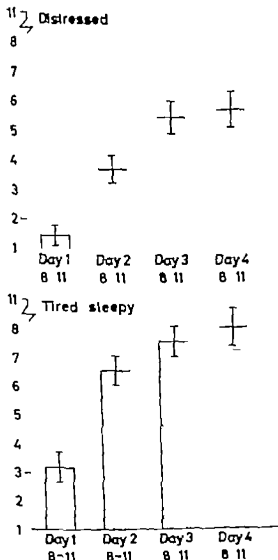
As shown in figure 7 2, magnitude estimations of "*distress*" increased significantly throughout the study A comparison of initial control levels (i.e. 8.00—11 00 a.m or 11 00 a.m.—2.00 p.m., on day 1) and levels during the corresponding periods of days 2, 3 and 4 shows a significant and progressive increase, see figure 7 3 Self-ratings on the 11-point scale (figure 7 4) show the same trend, though the highest means never reach more than moderate levels.

Magnitude estimations of "*fatigue*" increased step-wise from day to day throughout the study (figure 7.5), besides exhibiting a significant circadian rhythm, cf also figures 7 3 and 7 4

7 4.1.2 Observed behaviour

Pronounced fatigue was the main *observable* behavioural reaction in our group, cf. figure 7 6. In spite of the highly uniform environmental stimulation, and the subjects' relative lack of cues as to the hour of day sleepiness was observed to be far more pronounced during the hours immediately following midnight. It is noteworthy that three out of the four subjects who vomited did so during the early morning hours.

In general, the behaviour of our group was not



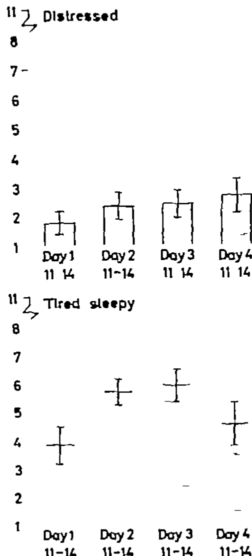


Figure 7.4 Self-rated distress and "fatigue (11 point rating scales) during the control period (day 1, 08—11 hours for half the group, 11—14 hours for the other half) as compared with the corresponding periods of days 2, 3 and 4. Means ± S.E.M. For description of rating scale, see p. 44

very conspicuous. *However rather pronounced* confusional reactions did occur in two subjects

Subject *A* was in his early twenties. On the third day in his short conversation during the food b eaks, he reported some confusion about what his fellow subjects said and also about some of his own viewpoints. He said, for example, that his thoughts spun clockwise" and that he ought not to forget the instruments for the dentist At noon, the experimenter observed that the subject was fiddling about with his rifle, scrutinizing it thoroughly but not shooting. *He related* that he did not know how to use it but was trying to find out. On being asked to leave the shooting range, the subject went to the wall, which he inspected and fingered in a search for non-existing seams. He was escorted to a bed and put to sleep Initially he exhibited some uneasiness about being cranky and therefore not being allowed to go home after *the end of the vigil. Being* reassured that such feelings might be normal in sleep-de prived people, he fell asleep and slept for 9 hours,



Figur 7·5 Self-rated "fatigue (magnitude estimations) under conditions described in figure 7.2. Means ± S.E.M.



Figure 7·6. View of the shooting range during early morning hours of the second night, illustrating behaviour characterized by pronounced fatigue and sleepiness. Picture taken with ultra-red (invisible) light and special film. The visible faces have been retouched to make them unrecognizable.

being woken up however every third hour for eating, drinking and producing a urine sample. Afterwards he reported no recollection of this sleeping period and also indicated that he had a complete blackout for the period just prior to his removal from the shooting range. Having slept, the subject recovered completely and finished the study as originally planned without behavioural or subjective disturbances.

The other subject, *B*" was in his late twenties. During the afternoon of the second day he reported increasing anxiety malaise and emotional tension. Indicating a wish to leave the study he was kindly asked to give it another try for a few more hours, which he agreed to do. After an hour or so he vomited and then reported that he felt better After a few more hours he suddenly rose, complaining of intense anxiety and claustrophobia, and indicated that he could not stand it any more He was immediately removed from the shooting range and put to bed, being dealt with in much the same way as subject A After 9 hours of sleep (with breaks every three hours for food, drink and urine samples) he felt completely recovered and finished the rest of the study without any subjective or observable disturbances.

7 4.1.3 *Performance*

Performance as reflected in *number of shots* and *number of hits* decreased significantly throughout the study cf. figures 7 7 and 7 8 Although the quality of performance (number of hits) varied considerably between any two consecutive 3-hour periods (probably mainly because of the differ ence in illumination between alternate periods) there is a tendency towards a circadian rhythm.

Briefly then, the exposure was accompanied by moderate increases in distress" and "fatigue save in a few subjects, who exhibited more pronounced reactions including confusional states, and by rather marked drops in performance.

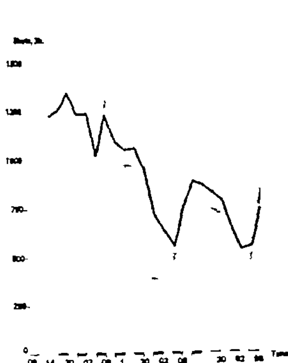


Figure 7.7. Number of shots per 3-hour period under conditions described in figure 7:2. Means ± S.E.M.

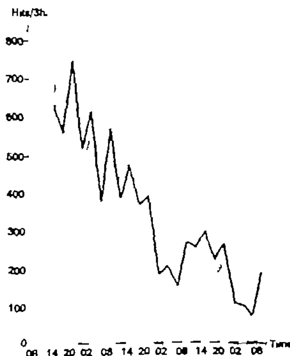
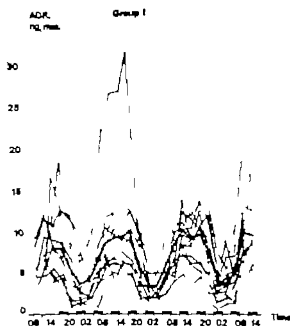


Figure 7.8. Number of hits per 3-hour period under conditions described in figure 7:2. Means ± S.E.M.



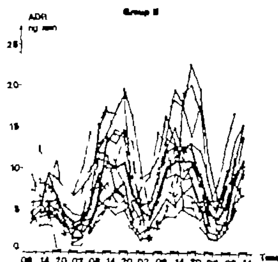
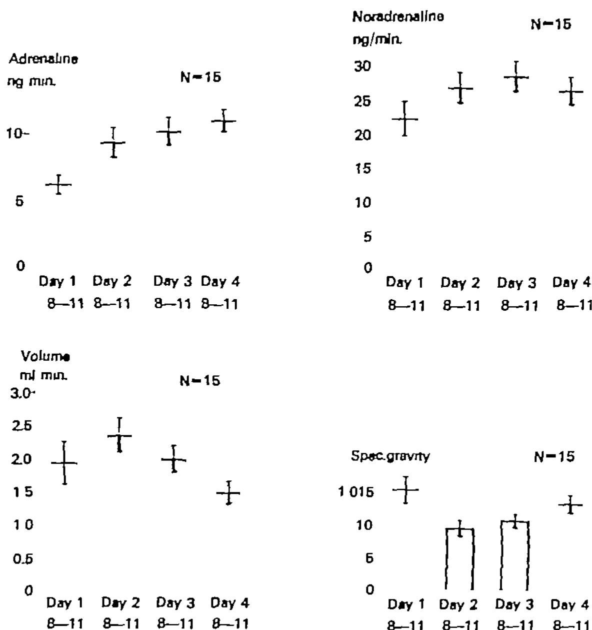


Figure 7.9. Adrenaline excretion under conditions described in figure 7.2. Adrenaline excretion peak in left diagram parallels the development and exacerbation of episode of claustrophobia and panic in one subject.



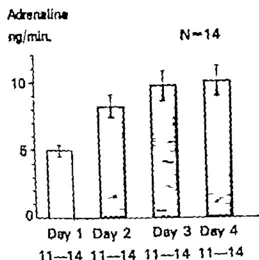
Figur 7.10 Urinary adrenaline and noradrenaline excretion, urine flow and specific gravity during control conditions (empty bars) and corresponding periods of days 2, 3 and 4, at 8—11 hours. Means ± S.E.M.

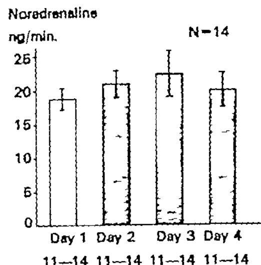
7.4.3 Physiological reactions

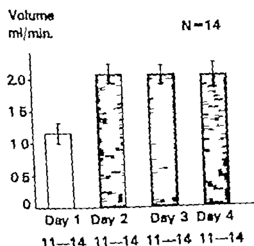
7.4.3.1 Adrenaline excretion

The urinary excretion of *adrenaline* followed a sine shaped curve, exhibiting significant circadian rhythm (Fröberg et al 1970) with a maximum during the hours following 12.00 noon and a minimum about 1 hours later cf figure 7·9 Adrenaline excretion exhibited a successive rise from the first day to the next, the increase over the control level being highly significant, figures 7 10 and 7 11

As shown in figure 7 9 one of the subjects exhibited a pronounced increase in adrenaline excretion on the second day of exposure. This was subject B who reported intense anxiety and claustrophobia (see above) and the increase in







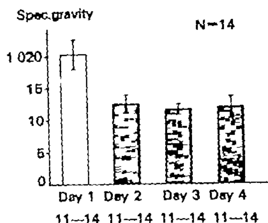


Figure 7.11 Urinary adrenaline and noradrenaline excretion, urine flow and specific gravity during control conditions (empty bars) and corresponding periods of days 2, 3 and 4 at 11—14 hours. Means ± S.E.M.

reported emotional tension largely coincided with the increase in catecholamine excretion, cf. also figure 7.1.

7.4.2.2 *Noradrenaline excretion*

Figure 7.12 demonstrates the individual levels of *noradrenaline* excretion throughout the study. The noradrenaline curve is more irregular than the one for adrenaline, exhibits a significant but less pronounced circadian rhythm (Fröberg et al., 1970) and shows peak values at about 8.00 a.m. Figures 7.10 and 7.11 demonstrate a significant rise from day 1 to day 2, but only for the period 8.00—11.00 a.m.

7.4.2.3 *Urine flow*

In spite of the 3-hourly standard intake of fluid throughout the experiment, *urine flow* exhibited

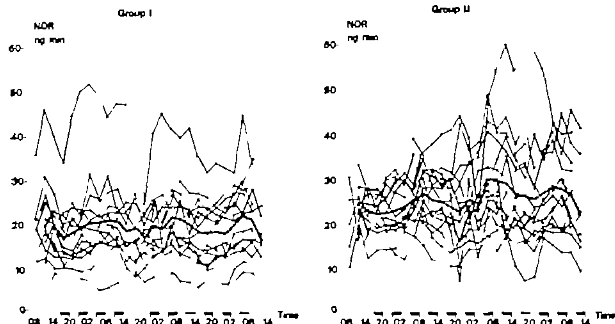


Figure 7.12 Noradrenaline excretion under conditions described in figure 7.2. Noradrenaline excretion peak in left diagram parallels the development and exacerbation of episode of claustrophobia and panic in one subject.

a very marked circadian rhythm (Fröberg et al., 1970) reaching peak values at about 9.00 a.m.

As to changes over time, the effects on this variable, as on several of the others, differ somewhat, depending upon which set of 3-hour periods the comparison concerned, namely 8.00 a.m.—11.00 a.m. or 11.00 a.m.—2.00 p.m., cf. figures 7.9 and 7.10.

7.4.2.4 Specific gravity

Specific gravity of the urine samples exhibited a decrease, cf. figures 7.10 and 7.11. Specific gravity displayed much the same cycle as urine flow but varied in the opposite direction.

7.4.2.5 Protein-bound iodine

The mean level of *protein bound iodine* and the S.E.M. the week before the vigil started was 6.1 ± 0.2 μg/100 ml plasma. At the end of the vigil, the level had risen by 30 per cent to 7.9 ± 0.2 μg/100 ml plasma (figure 7.13), the rise being statistically highly significant ($p < 0.001$), cf. Johansson et al. (1970).

In the "pre-stress" samples, PBI levels above 8 μg/100 ml plasma (i.e. above the upper normal limit with the assay method used in this context) were found in three subjects. At the end of the exposure, eight of the 31 subjects exhibited levels of this magnitude.

7.4.2.6 Serum iron

The "pre-stress" *serum iron* level was 111 ± 6.1 μg/100 ml plasma. At the end of the vigil the level had decreased by 52 per cent to 53.0 ± 3.7 μg/100 ml plasma ($p < 0.001$), see figure 7.14.

In the "pre-stress" samples, serum iron levels below 75 μg/100 ml plasma (= lower normal limit) were found in three of our subjects. At the end of the exposure, levels below this point were found in 28 subjects.

7.5 Discussion

7.5.1 Self-ratings, observed behaviour and performance

7.5.1.1 Self-ratings

As shown above, self ratings of "distress" and "fatigue" increased significantly throughout the exposure. It is noteworthy that the enhancement of "distress" and "fatigue" ratings over time is

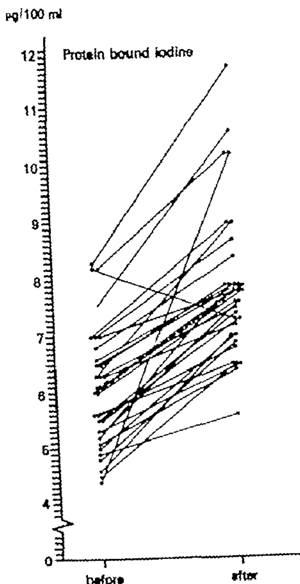


Figure 7.13 Protein-bound iodine before and after the stressor exposure.

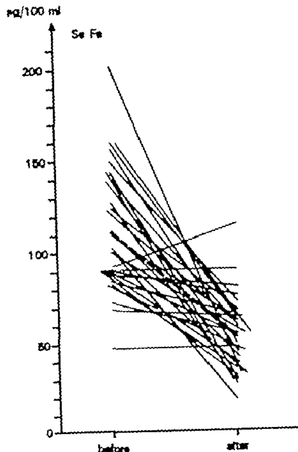


Figure 7.14 Serum iron before and after the stressor exposure.

more pronounced when the comparisons between days are made with respect to the period 8.00—11.00 a.m. than to the next period, 11.00 a.m.—2.00 p.m., cf figures 7 3 and 7 4 This finding supports the assumption of an interaction between duration of exposure and time of day as influenced by a circadian rhythm. The 24-hour means for each of the three days likewise show significant increases in "distress" and "fatigue" ratings (Fröberg et al., 1970)

7.5.1.2 Observed behaviour

Dramatic delusional and confusional states have been described repeatedly in sleep-deprived subjects (for review see Naitoh, 1969 Rechtschaffen and Kales, 1969). It has been claimed that sleep-deprived subjects who develop florid psychotic behaviour appear to have atypical personalities and personal histories. However the two subjects in the present study who exhibited a confusional and a claustrophobic reaction, respectively were not and had never been psychiatrically conspicuous as judged from pre-experimental psychiatric interviews. Their reactions were rather benign and transient, disappearing after the equivalent of a good night's sleep

7.5.1.3 Performance

Performance decrements following sleep deprivation combined with continuous work have been described repeatedly (for review see Naitoh, 1969 Morgan et al 1970 Drucker et al 1969) Our results, indicating quality and quantity decrements, are in general agreement with those reported by other authors. Feeding back information to the subject on how well he had performed each task shortly after its execution (Wilkinson, 1961) and the intermittent exposure to noise (Wilkinson, 1963) probably tended to counteract the performance decrements. The reasons for the decrements are no doubt manifold, one of them probably being the occurrence of brief intermittent lapses in performance as described e.g. by Bjerner (1949), who demonstrated that such lapses were accompanied by a transient fall in pulse rate and a change in the EEG pattern, indicative of brief periods of sleep.

The quality and quantity of performance are no doubt influenced by many other factors as well, such as changes in motivation (cf. Ax et al., 1957) and the occurrence of minor neurological disturbances, e.g. ptosis and tremor (Sassin, 1970) It is unlikely that learning contributed to counteract the performance decrements substantially because all the subjects were qualified marksmen and the shooting task was not very different from ordinary rifle shooting.

7.5.2 Physiological reactions

7.5.2.1 Adrenaline excretion

As shown in the review in paragraph 1.5.2 and in Chapters 3—6, psychosocial stimuli of short duration can evoke enhanced sympathoadrenomedullary activity as reflected in plasma levels and urinary excretion of adrenaline. The present study demonstrates that a similar reaction occurs in response to a stressor exposure lasting several days. The prolonged exposure is accompanied by a significant and sustained increase in adrenaline excretion over control values (cf figures 7 10 and 7 11) as well as by a significant increase in 24-hour adrenaline excretion over days (Fröberg et al., 1970)

Rubin et al. (1969) reported a 205 hour sleep deprivation study on 4 subjects, 2 of whom exhibited increased excretion levels of urinary vanillylmandelic acid (VMA) during the latter half of the deprivation period. However these subjects had access to television, radio, a phonograph, table tennis, and a snack kitchen, and in the slack periods between various psychological testing procedures they amused themselves with games, physical activities, and other diversions. In addition, according to the authors, the subjects used a basin of ice cubes-face immersion technique to combat increasing waves of drowsiness, creating their own version of the cold pressor test, which has been shown to increase the urinary excretion of VMA (Sapira and Shapiro, 1966), making the results difficult to evaluate. Exposing subjects to physical work in combination with ambient temperatures and one night's sleep deprivation, Hasselman et al. (1960) reported that the last-named factor contributed to the increase in sympathoadrenomedullary activity thus provoked (cf. also Hernández Peón et al. 1969). Florica et al. (1970), on the other hand, found no increases in "total catecholamine" excretion.

7.5.2.2 Noradrenaline excretion

Noradrenaline excretion generally remained on the control level and did not exhibit pronounced fluctuations attributable to the duration of exposure. It may be noted, however that in subject B who developed an episode of claustrophobia and panic, noradrenaline excretion rose to and remained on rather high levels before and during this episode.

7.5.2.3 Urine flow and specific gravity

As to urine flow and specific gravity an increase in diuresis was found during the stressor exposure as compared to the initial control periods. This is consistent with our findings in most of our previous experiments. On the other hand, at least part of this response probably reflects rather high hydration, 300 ml of water being ingested every third hour Although this fluid dosage began prior to the study proper it is conceivable that water balance was not reached until well after the start.

The 24-hour means for urine flow showed a significant increase from day 1 to day 2 (Fröberg et al., 1970).

7.5..4 Protein-bound Iodine

As demonstrated in figure 7 13 our subjects exhibited a highly significant increase in PBI, sometimes even exceeding the upper normal limit.

For a more comprehensive discussion of these findings the reader is referred to Johansson et al (1970). In the present context we shall simply consider some of the main issues.

The first question is whether the PBI rise was caused by the combination of continuous shooting and sleep deprivation, as opposed to stimuli like the 3-hourly food servings. This hypothesis might have been tested by letting a control group just sit and be exposed to the experimental procedures described above, excluding, however the sleep deprivation and the psychomotor activities. Such a prolonged non-stress study was, however considered unsatisfactory as the sheer monotony would presumably represent a stressor

PBI levels have been shown not to change appreciably over relatively short periods of time (Danowski et al., 1949), even in the *absence* of any standardization of diet, physical and mental activities etc. Similarly Gaffney et al. (1960) report that an increase in the interval between PBI measurements from 1—13 days to 13 days—7 months did not increase the variability in level.

Other reasons, too, make it unlikely that the present diet contributed to the rise in PBI. With an adequate dietary supply of iodine, an additional daily ingestion of up to 125 mg of iodine is reported not to alter PBI levels appreciably (Friend, 1960) Such an amount corresponds to no less than 3 kg of iodized salt, i e much more than any one could possibly ingest over a period of a few days. Neither did the subjects in fact receive appreciable amounts of iodine from non-dietary sources. Had the subjects started with an iodine deficiency however it is conceivable that even mor or less normal" doses of iodized table salt would have increased the levels of PBI (cf. Kelsey et al., 1957). But in our case, iodine deficiency (cf Lundwall et al., 1965) at the start of the study is improbable, because the diet used in the Swedish Army (not comprising iodized salt) has been calculated (Karlsson and Levi, 1969) to include approximately 120 μg of iodine daily This amount compares favourably with the recommended daily iodine allowance for adult males, which is 110—140 μg (U.S National Research Council s Food and Nutrition Board 1968) Furthermore, the sandwiches served in our experiments cannot have constituted even a modest iodine load" as mentioned above, because *none* of their ingredients is prepared with iodized salt or contains iodine in appreciable amounts (cf Karlsson and Levi, 1969) Accordingly the PBI rises in our studies could not be attributed to dietary factors of the type described above

On the other hand, it is of course impossible to separate the psychosocial from the physical stimuli included in the experimental situation, the latter being the prolonged sitting, the monotonous and oft repeated ingestion of food etc. (cf. Bergner et al., 1968) Suffice it to say that exposure to the total experimental situation, including as it did several conspicuous psychosocial elements, resulted in significant increases in PBI levels.

Our second key question relates to whether or not the rise in PBI is accompanied by a corresponding increase in thyroid activity and in free thyroxine in plasma.

Protein-bound iodine normally consists mainly of thyroxine, small amounts of triiodothyronine and perhaps traces of other iodinated substances as well (for a review and discussion see Wayne et al., 1964). As a rule, PBI is considered to be an approximate measure of the concentration of thyroid hormones in the blood (Scazziga and Lemarchand Béraud 1967 Mason 1968, Farran et al., 1971). As often pointed out, this measure is not a simple function of the rate of hormonogenesis but merely a steady state concentration (Gregerman, 1967).

This steady state may be disrupted in several ways, as demonstrated by LaRoche and Johnson (1967) in rats exposed to a simulated altitude of 17,000 feet. The authors suggest "than in the early stages of treatment there is a profound dichotomy between rates of thyroidal uptake and

secretion. Similarly Söderberg (1958) concludes that rate of secretion and rate of uptake are not parallel in the acute experiment. *If this is true in* man, the rise in plasma PBI found in our studies may theoretically have been determined not only by an increased thyroxine release (cf. Dewhurst et al., 1968 b) or by a (catecholamine induced?) shift in the compartmental distribution of hormonal iodine (Hays and Solomon, 1969) but also by a decreased elimination rate, including decreases in some or all of the following processes. (a) peripheral utilization, (b) tissue binding, and (c) excretion (Brockis 196_).

However the demonstration by Blomstedt (1965) of an increase in thyroxine breakdown following surgical trauma as well as cortisone administration argues against such a decrease in thyroxine elimination rate. Surgical trauma has further been reported to result in an increased production of thyroxine as measured by changes in the protein-bound iodine" as well as in a reduced uptake of iodine by the thyroid immediately after the operation (Johnston, 1965). The levels of protein-bound iodine rose quickly and were maintained for 3 days after operation. The author interprets his results as pointing to a redistribution of iodine in the body after injury (with less iodine a ailable to enter the thyroid gland) and to a rapid release of preformed stored thyroid hormone after trauma, along with an increased rate of hormone utilization by the tissues. Studying the same phenomenon, Brockis (1962) reports findings which suggest that active changes take place in the thyroid gland following a surgical operation, and as it is known that the content of iodine in urine is not diminished under these circumstances, this could not account for the rapid rise in hormone level. In an analogous way the thyroid of normal man is said to respond rapidly to acute febrile illness with a marked increase in the rate of release of *thyroxine (Gregerman, 1967) However as the* bacterial illness often results in a marked and perhaps proportional acceleration of thyroxine elimination ($t_{1/2}$ for injected thyroxine I^{131} decreasing from 10.8 to 2.3 days), the plasma thyroxine concentration may remain unaffected (Gregerman and Solomon, 1964)

As judged from these studies there is no a priori reason to expect that thyroxine *elimination from blood should decrease during the stressor* exposures of our study. If anything, it could be expected to increase because (a) the urinary excretion of iodine has been shown to increase following catecholamine injection in the rat, rabbit and dog (cf. Pitt Rivers, 1960) and (b) as indicated above, an increase in tissue utilization has been proposed to occur in response to various stressor exposures, possibly as a result of the increased release of catecholamines (cf. Pitt Rivers, 1960). As all these processes tend to lower the PBI level, and yet the PBI levels rose in our studies, our results speak in favour of a thyroxine release big enough to make up for a presumably increased elimination and, in addition, to raise the plasma pool of circulating hormone.

Determinations of the level of untagged PBI in contrast to PBI^{131} and radioactivity counted over the thyroid gland did *not* change significantly according to Flagg et al. (1965) in subjects exposed to a stressor film, suggesting that untagged PBI is not a particularly sensitive indicator of thyroid response. On the other hand, we considered it inexpedient to use radioiodine methods in large-scale studies with healthy volunteers. Furthermore, as pointed out by Brockis (1962), the tracer protein-bound iodine represents only a small proportion of the overall protein-bound iodine. The increases and decreases reflect the fate of the most recently produced thyroid hormone. Whether this protein-bound iodine fraction behaves similarly to the overall PBI is not yet known.

High PBI levels are occasionally found in patients with clinically normal thyroid function (cf. Rosenbaum et al. 1968). What remains normal is the small non-protein-bound or *free* thyroxine fraction, which represents the physiologically *active form of this thyroid hormone. The high* PBI levels in such a case may be due to increased levels of thyroxine that has been *bound by serum proteins*, particularly thyroxine-binding globuline (TBG) but also thyroxine-binding albumin and prealbumin (TBPA) (Sisson, 1965 Scazziga and Lemarchand-Béraud, 1967). If the number of

available binding sites of TBG and (to a lesser extent) TBPA and albumin increases, more thyroxine becomes bound to these sites and the PBI level rises. This is said to occur during pregnancy, estrogen administration (including the common oral contraceptives), prolonged perphenazine administration, and acute liver disease and cirrhosis (Sisson, 1965). However, none of these influences is very likely in our officers and soldiers, all of whom were in perfect health as judged by clinical criteria and not on any drug regimen whatsoever.

Furthermore, surgery has been reported to induce not an increase but a significant decrease in the binding capacity of TBPA, a fall in prealbumin-1 (Surks et al., 1967) and in TBPA (Surks and Oppenheimer, 1964) and an increase in the peripheral breakdown of thyroxine (Blomstedt, 1965). Further, the release of corticosteroids is known to rise during stress (Mason, 1968), and cortisone derivatives have been reported to reduce the binding capacity of TBG (Scazziga and Lemarchand-Béraud, 1967). Against this background it is considered improbable, although definitely not impossible (cf. Taylor and Fisher, 1968; Hays and Solomon, 1969), that the PBI increases found in our study were due predominantly to changed levels of carrier proteins.

This position finds further support in the recent demonstration by Crystal et al. (1970) of a rise in free thyroxine and a fall in TBPA occurring 1—4 days after myocardial infarction in five euthyroid subjects.

As already noted, three of our subjects exhibited "pre-stress" PBI levels above 8 μg/100 ml plasma. It is not known whether these subjects experienced specially pronounced anticipatory anxiety or whether their rather high PBI levels were due to other factors. Anyhow, none of them was clinically hyperthyroid. The mean "pre-stress" PBI level of our group was, however, quite normal (6.1±0.2 μg/100 ml) and rather close to the normal levels obtained with the same method (5.8±0.4 μg/100 ml) and reported by Lemarchand-Béraud and Vanotti (1969).

To sum up, we managed to induce relatively pronounced and highly significant increments in PBI levels in our group of 31 young and middle-aged military subjects. It is not entirely clear whether these increments were due to rises in biologically inactive thyroxine, indicating a rise in carrier proteins, or whether they reflect either an increase in thyroid activity accompanied by increased levels of free thyroxine, or an adrenaline-induced shift of thyroxine from tissue stores into the vascular compartment.

In a subsequent study of similar design, significant PBI increments were induced in 32 senior officers (Levi, 1969; Johansson et al., 1970), confirming the present results. In a third study to be carried out this year, the mechanisms of the PBI rises will be investigated in more detail.

7.5.2.5 Serum iron

As indicated above, the stressor exposure was accompanied by a pronounced and highly significant fall in serum iron, in fact down to levels usually considered clearly subnormal, in spite of a rather liberal supply of iron-containing food throughout the study. This dramatic drop in serum iron could not be due to the venous punctures per se, as the blood loss amounted to no more than 50 ml per puncture. Liljedahl et al. (1969), in their studies of plasma iron elimination rate, found that phlebotomy of 100—150 ml did not accelerate this elimination.

The "pro-stress" serum iron levels of our group were very close to those found for healthy Swedish adult males as reported in numerous papers (for a review, see Strandberg, 1966). In contrast, as indicated above, the "post-stress" serum iron levels of all but 3 subjects were below the lower limit of the normal range (cf. Fairbanks, 1970).

As mentioned in paragraph 7.1, serum iron levels have been shown to decrease in response to a variety of physical stressors and in the course of diseases as diverse as rheumatoid arthritis, cancer, infections, and mental illness. This brings to mind the Selye hypothesis of stress as the non-specificity of physiological response. The present finding speaks in favour of this hypothesis, demonstrating that the stressor exposure of the present study, too, evokes a serum iron decrease.

Our results have been confirmed in a similarly designed study on 32 senior officers (Levi, 1969).

and in studies reported by Kuhn et al. (1967 a and b). These authors found the same type of serum iron decrements in 4 males exposed to a 120 hour vigil and confirmed their findings in a second study on another group of 6 males. During sleep deprivation they found a gradual decline in serum iron, the maximum drop being to half the original levels, in much the same way as in our study The decline was most marked during the first 48 hours of sleep deprivation. The return to normal values took roughly one week. Total iron binding capacity also decreased, but much less, the maximum drop occurring during the 72nd hour of the vigil. Urinary iron excretion and gastrointestinal iron absorption did not change significantly

In agreement with the last named authors we are inclined to interpret these results along the lines proposed for the serum iron decrements found in response to physical stressors, namely with reference to a stimulation of the reticulo-endothelial system, probably induced through neuroendocrine pathways. However future studies should also consider other theoretically possible explanations, such as an increase in hemoglobin synthesis and a decrease in hemolysis and/or in iron release from tissue stores. No doubt, the problem deserves further study

7.5.2.6 Hematocrit

Before drawing any conclusions as to changes in various plasma and serum constituents, one must consider the possibility that the stimuli evoked changes in the extracellular fluid volume, which, in turn, would automatically change the concentration of all compounds normally present in this fluid. Distress reactions are known to be accompanied by changes in water and electrolyte metabolism (Schottstaedt et al., 1956) The resulting shifts of water between intra and extracellular fluid compartments may conceivably influence the serum level of various compounds, e.g. serum iron and PBI (Reichlin and O'Neal, 1962 Wilson, 1966) If the extracellular fluid volume decreases, the concentrations of these compounds may be expected to rise, and vice versa. Such a hemoconcentration or hemodilution, if pronounced, would be reflected by the hematocrit. However no significant changes in hematocrit were found in our study the "pre-" and "post-stress levels being 43.9±0.5 and 43.0±0.5 respectively ($p > 0.05$).

It is an everyday experience that prolonged sitting leads to swelling of the feet. This phenomenon was noted in several of our subjects towards the end of the exposure. As shown by Johnson et al. (1972) 24 hours of sitting, although leading to swelling of the lower extremities, does not evoke any change in interstitial and intracellular fluid volumes as calculated from measured plasma volume, extracellular volume and total body water of 6 subjects before and after a 4-hour commercial overseas flight. Neither did peripheral hematocrit or total serum protein concentration change significantly It seems unlikely that the shift in body fluids to the dependent parts of the body explains the changes in serum levels of various compounds, as there is no water retention and the intravascular water volume remains unchanged.

7.5.3 Circadian rhythms

Briefly all our indices of sympathoadrenomedullary and renal function as well as our fatigue and distress ratings exhibited circadian rhythms during most of the 24 hour spans, in spite of the uniform nature of the routine and the extensive equalization of extrinsic Zeitgebers (task, ingestion of food and fluid, bodily posture etc.) between all 3-hour periods of the day (Fröberg et al., 1970). The quantity and quality of performance decreased, and distress and fatigue increased during the early morning hours (3 00—5.00 a.m.). It may be noted that these reactions did not coincide with, but were *preceded* by a pronounced drop in adrenaline excretion. This sequence of events may be a cue to a possible causal relationship between the two sets of variables.

Several of the psychophysiological circadian rhythms may have obvious implications in situations where subjects are expected to perform continuously for 24 hours or more, or to alternate

their periods of work and sleep as in shift work (cf. Lnce, 1970).

It has further been shown that births (Jenny 1933 Málek et al., 1962, Kaiser and Halberg, 1962) and deaths (Frey 1929 Jusatz and Eckardt, 1934) reach their peak frequencies and several somatic diseases make their début or exacerbate (cf. e.g. Menzel, 194 Halberg, 1953 Zülch and Hossman, 1967 Ask Upmark, 1969) at the very hours when, according to Shakespeare (1605), churchyards yawn, and hell itself breathes out/ Contagion to this world now could I drink hot blood/ And do such bitter business as the day/ Would quake to look on" i.e. during the hours round about midnight, whereas e.g. endogenous depressions usually are reported to be most intense not until the early morning hours, when depression comes down like a cloud" (Slater and Roth, 1969 Middelhoff 1967). It would be tempting to speculate here about this time lag between physiological and psychological phenomena which obviously occurs in clinical practice as well as in our experimental situation, but the *circadian aspects* of the present study and others conducted at our laboratory will be dealt with in detail in a future report (Fröberg, in preparation)

7.3.4 Psychophysiological relationships

Considerable numbers of parallel psychological and physiological data were obtained concurrently in the present study which therefore constitutes a better basis for the assessment of psychophysiological relationships than do the studies reported in Chapters 3—6

Correlations were computed over the entire sequence of the last 16 3-hour periods, based on group and period means for each pair of variables. In this analysis the observations from the first 24 hours were excluded in order to diminish possible effects of learning the task and getting acquainted with the situation, which would presumably complicate the relationship It was found that both performance measures correlated positively and significantly to adrenaline excretion but negatively and significantly to noradrenaline excretion, cf. table 7 1

Table 7 1 Correlations between paired means of variables calculated for the last 48 hours of the experiment.

Variable A	Variable B	r_{AB}	d.f	p<
Adrenaline	Noradrenaline	05	14	
	Urine flow	.31	14	
	Shots	66	14	01
	Hits	.55	14	.05
	Fatigue	–.54	14	05
	Distress	–.24	14	
Noradrenaline	Urine flow	73	14	001
	Shots	– 52	14	.05
	Hits	– 54	14	05
	Fatigue	.53	14	05
	Distress	.55	14	.05
Shots	Fatigue	– 92	14	001
	Distress	– 78	14	.001
Hits	Fatigue	–.87	14	.001
	Distress	–.86	14	001

The reverse was true regarding the correlations between self-ratings of fatigue and urinary catecholamines. Thus, fatigue ratings correlated significantly but negatively with adrenaline excretion, and significantly and positively with noradrenaline excretion.

There was also a significant positive correlation between distress ratings and levels of noradrenaline, whereas the correlations with performance measures (shots and hits) were significant but negative, cf table 7 1

Similarly performance showed high negative correlations with fatigue ratings.

When computing correlations between variables, it must be kept in mind that the reactions, although simultaneously *measured* did not necessarily *occur* simultaneously but can still be significantly *related* to each other As shown by Fröberg et al. (1970), minimum adrenaline excretion levels *precede* by several hours the subsequent low in performance and high in fatigue ratings. Therefore part of our analysis has been based not only on a correlation of data obtained in our subjects from the same periods of measurement but also on correlations obtained after *lagging* one set of the data 1—2 measurement periods. The choice as to which variable to lag was based on inspection of the curves and calculations con-

Table 7 2 Correlations with 3- and 6-hour lags between paired means of variables. A lag of e. g. 3 hours for a variable implies that the values for that variable have been "shifted" one measurement period in relation to another variable.
Calculations refer to the last 48 hours of the study

Variable A	Variable B	Lag in hours	Lagged variable	r_{AB}	d.f	p<
Adrenaline	Noradrenaline	3	B	.55	13	.05
		6	B	76	12	01
	Urine flow	3	B	.82	13	.001
		6	B	70	12	01
	Shots	3	A	78	13	.001
		6	A	40	12	
	Hits	3	A	65	13	01
		6	A	.28	12	
	Fatigue	3	A	− 75	13	01
		6	A	−.50	12	.05
	Distress	3	A	− 44	13	
		6	A	−.32	12	
Noradrenaline	Urine flow	3	B	49	13	05
		6	B	19	12	
	Shots	3	B	−.80	13	.001
		6	B	−.81	12	001
	Hits	3	B	− 63	13	05
		6	B	− 81	12	001
	Fatigue	3	B	83	13	001
		6	B	69	12	.01
	Distress	3	B	.53	13	05
		6	B	.52	12	.05

-erning their phases (Fröberg et al., 1970) Some f the results of these analyses are shown in tabl 7 2.

It will be seen from this table that several correlation coefficients become considerably higher if one of the variables is lagged one or two 3-hour periods (cf Fröberg et al., 1970)

In describing the relationships between different sets of variables, one may further wish to separate two important factors, both of which may influence the correlations under study·

(a) progressive changes due to sleep deprivation etc. per se, and (b) circadian variations.

Making the assumption that the former effects are approximately linear with time, partial correlations were computed (Fröberg et al., 1970) The results of this analysis indicate that when the effect of hours of sleep deprivation is "partialled out the correlations between *adrenaline* excretion on the one hand and performance and fatigue measures on the other are clearly significant and of the same magnitude as those reported in table 7 1 (r = .80 for number of shots, and − 70 for fatigue ratings) The corresponding correlations between adrenaline excretion and distress ratings was − 19 Partial correlations between *noradrenaline* and performance, distress and fatigue, however were not significant. These results support the assumption that there is a relationship between the *rhythms* of adrenaline on the one hand and performance and fatigue on the other while the relationship between the two latter variables and noradrenaline excretion is due primarily to stressor induced" progressive changes. In evaluating the non significant, negative correlations between adrenaline excretion and distress ratings (table 7 1) it should be kept in mind that distress and fatigue ratings were highly positively intercorrelated in the present experiment (r = 78, p 01) robably most of the distress, which mo n rather modest levels, u

only experienced. There is probably a considerable qualitative difference between the distress reported in several of the previous chapters and e.g. in studies conducted by Frankenhaeuser (1971) on the one hand and the present distress ratings on the other. The situation was neither open-ended nor obviously threatening. With the exception mentioned above, no manifest anxiety was observed or reported. Probably a high fatigue loading in the distress ratings accounts for the negative correlations found between adrenaline and distress.

7.5.5 Miscellaneous physiological variables

As indicated by the data presented above, the stressor exposure was accompanied by a number of significant physiological reactions, some of which are significantly related to performance and/or self ratings of distress and fatigue. The assumption that the stressor exposure did, indeed, significantly influence physiological function is further supported by the finding of significant increases in erythrocyte sedimentation rate (from 2.8±0.4 to 7.5±0.9 mm per hour, $p <$ 0.001). A more recent study on senior officers demonstrated the same trend, accompanied by ST and T level depressions in the ECG, decreases in fibrinolysis, and increases in plasma free fatty acids and cholesterol (cf. Levi, 1972). Some clinical implications of these findings will be discussed briefly in the next chapter.

7.6 Summary

A study is presented, in which a total of 31 Army officers and corporals were exposed to a 75-hour vigil that started with a 3-hour control period and continued with 72 hours of performance on an electronic shooting range under strictly standardized environmental conditions. It was found that the exposure was accompanied by significant and pronounced increases in fatigue ratings, moderate increases in distress ratings, pronounced but transient confusional reactions in two of the subjects and decreased psychomotor performance. Simultaneously, our subjects exhibited marked and significant increases in adrenaline excretion and in protein-bound iodine, and a marked and significant decrease in serum iron.

7.7 Acknowledgements

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8 GENERAL DISCUSSION

By Lennart Levi

8.1 Objectives of this chapter

The data presented so far have been discussed in some detail, but only chapter by chapter. In the present chapter the theoretical model and some of the main hypotheses presented in Chapter 1 will be discussed, drawing on data from several studies, including those by other authors. Hypotheses and data discussed in earlier chapters will either not be repeated at all, or only in summary form.

Some of our findings seem to have implications for clinical and occupational medicine, and for social planning and engineering. A few of these implications will be presented and discussed.

Some of our findings also make possible the formulation of new hypotheses and proposed routes for further research. Based on these considerations, some general outlines for a future research program will be presented.

8.2 Psychophysiological reactions to psychosocial stimuli

8.2.1 Psychological response to psychosocial stimuli

As mentioned in paragraph 2.17, coping behaviour and differences in attitudes can profoundly modify human responses to psychosocial stimuli. Accordingly, it can never be taken for granted that an experimental or real-life situation will evoke distress and stress merely because the experimenter assumes this to be the case. Therefore, before stating anything about psychophysiological relationships, the experimenter is obliged to check, by questionnaires, interviews or behavioural observations, whether the subjects did in fact react as predicted. For example, the present author assumed, for obvious reasons, that the sex films (Chapter 4) would induce at least some degree of sexual arousal, yet one out of five of the female subjects denied even the slightest arousal of this type. Although we do not know for certain whether these self-ratings reflected the actual degree of sexual arousal, the ratings do have some face value, and the finding illustrates the necessity of not using situational criteria alone but of also always checking with the subjects concerning their subjective reactions to the stimuli applied.

As repeatedly emphasized, the simple ordinal rating-scales, although offering the advantage of simplicity and intelligibility, are no doubt rather crude yardsticks for measuring subjective reactions. They may be adequate when used for qualitative purposes only, as was the case e.g. in the studies reported in Chapters 3 and 5, but if we want to use them for quantitative purposes, e.g. primarily for the study of psychophysiological correlations, it is probably preferable to use methods like magnitude estimation, cf. Chapter 7. Using this method we were able to demonstrate distinct and reproducible maxima and minima as well as in-between sections of the fatigue self-rating curve (figure 7.5). The mere shape of this curve, and the rather high correlation between the fatigue ratings on the one hand, and performance and physiological variables on the other, speak strongly in favour of the application of this method of measurement in psychophysiological studies.

8.2.2 Psychosocial stimuli, physiological mechanisms, and disease

Paragraph 1.3 outlined some hypotheses within the general frame of reference presented in our theoretical model (figure 1.1). It was hypothesized that a variety of environmental psychosocial stimuli (e.g. "life changes", cf. Rahe, 1972) would elicit an increase in stress (Selye), characterized

inter alia by enhanced sympathoadrenomedullary activity and, concomitantly by increased lipolysis, under certain circumstances contributing to a hyperlipoproteinemia. These and other phylogenetically old adaptational reactions, usually rather obsolete in today's psychosocial environment, prepare the organism for a physical activity that is rather seldom manifested. Simultaneously with this prolonged state of physical "preparedness," the rate of wear and tear in the organism increases, as do eventually morbidity and mortality

Against the background of what has been reported in the previous chapters we will now reappraise some of the hypotheses on which our model is based.

According to our first hypothesis, psychosocial stimuli lasting *hours* or *days* evoke physiological reactions, comprising stress (Selye)

The results presented in this volume, comprising own studies as well as those by others (reviewed in Chapter 1) confirm this hypothesis. Short-term exposure to a variety of stimuli, in laboratory settings as well as in real life, has been shown to evoke increases in urinary and plasma catecholamines and 17-hydroxycorticosteroids, free fatty acids and triglycerides. At least the response in urinary catecholamine excretion seems to be highly non-specific, occurring in connection with a very wide variety of stimuli, as postulated in Selye's stress" concept.

Similarly a real work setting (lasting about 8 hours) and a semirealistic stressor exposure with 77 hours continuous work in a shooting range induce protracted enhancement of adrenaline excretion levels, and—in the last named study—increased levels of protein-bound iodine and decreased levels of serum iron. In addition, erythrocyte sedimentation rate increased, and ST and T changes occurred in the ECG A later study reviewed (but not presented) in Chapter 7 showed increases in free fatty acids and cholesterol and decreases in fibrinolysis.

A logical next question would be to ask whether psychosocial stimuli occurring over *weeks* and *months* would evoke physiological reactions, comprising stress (Seyle)

To elucidate this problem in relation to the data presented by Rahe and others (for a review see Rahe, 1972) the following study was conducted in collaboration with our laboratory (Theorell, 1970 Theorell et al., 1972)

Twentyone male, well rehabilitated survivors of a myocardial infarction gave weekly reports for 2—4 months of all major life changes that had occurred during the previous week. A life change unit sum was calculated according to Holmes and Rahe (1967) Urine samples were collected weekly during the day prior to these reports, under strictly standardized conditions. A positive and significant intra-subject co-variation was found between the weekly sum of the life change units and the pre interview day adrenaline output.

The results referred to above support the assumption that psychosocial stimuli of short or moderate duration, created in a laboratory or occurring in real life, all evoke changes in sympathoadrenomedullary activity possibly as part of a phylogenetically old, non-specifically evoked reaction pattern, stress (Selye)" inter alia often (or always) accompanied by increased lipolysis.

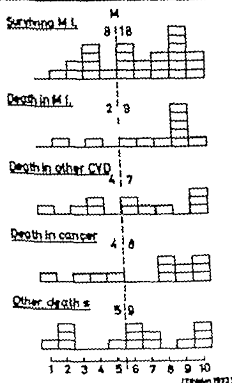
Accordingly a relationship no doubt exists between boxes 1 and 3 in figure 1 1 (see page 12)

Our next question is whether a corresponding relationship exists between psychosocial stimuli and disease (boxes 1 and 5)

Such a relationship can be specific (i.e. it relates to a particular disease) or non-specific (i.e. relating to a variety af diseases). To elucidate the first-named possibility Theorell (1970) studied the degree of life change to which subjects had been exposed, who approximately six months later developed *myocardial infarction.* He and other investigators (cf. Rahe and Lind, 1971 Rahe and Paasikivi, 1971 Rahe, 1972) found, indeed, that exposure to many and/or dramatic life changes was associated with a subsequent increase in morbidity and mortality in myocardial infarction.

Moreover as demonstrated by several authors, such an exposure seems to predict increased morbidity and mortality not only in myocardial infarction but in *other diseases* as well (for a comprehensive review see Rahe, 1972).

Turing back to our model (figure 1 1) let us



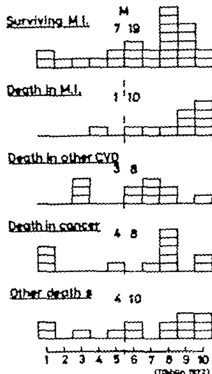


Figure 8.1 Plasma triglycerides and cholesterol as predictors of morbidity and mortality in myocardial infarction (M.I.), and of mortality in other diseases. Distribution in deciles (1—10) of plasma levels in relation to deaths. Vertical line indicates medians. Each box indicates one death or case of illness. Figures indicate distribution of below-median (left) and above-median (right) triglyceride and cholesterol levels. (Tibblin, personal communication.) Clearly above-median levels seem to predict subsequent morbidity and mortality.

now examine the relationship between physiological reactions (i.e. mechanisms) and disease (boxes 3 and 5). Again, this relationship, if any, can be specific (e.g. increased plasma triglyceride and cholesterol levels predict degenerative heart disease) or non-specific (i.e. the plasma levels of these compounds predict increased morbidity and mortality in a variety of diseases).

The first-named (specific) relationship, demonstrated by many authors, can be exemplified by the well known relationship between hyperlipoproteinemia and degenerative heart disease (cf. e.g. Keys et al., 1971). However, and more interesting, high plasma levels of triglycerides and cholesterol have also been shown to constitute a *non-specific* risk factor (induced by increased sympathoadrenomedullary activity?) predicting *mortality in general* (Tibblin 1972, personal communication), as demonstrated in a large-scale study of initially healthy males born in 1913 and followed annually since 1963 (see figure 8.1). The subjects formed a representative sample of men aged 50 in Gothenburg at the time of the biochemical assessment.

True, the *entire* sequence of events shown in figure 1.1 has never been demonstrated in man. On the other hand, attempts have been made, some of them successful, to demonstrate it in animals, including primates (for review, see Levi, 1971).

Briefly, then, evidence has been presented in favour of most of the links in the hypothetical chain of events comprised in our model. Admittedly much of the evidence is still no more than

suggestive. But the data fit the hypothetical pattern sufficiently well to justify future research in the area outlined above, e.g. by methods described in the present volume.

8.3 The "stress (Selye)" concept

As demonstrated in Chapters 3—7 psychosocial stimuli do clearly influence urinary catecholamine excretion, either enhancing or lowering it, depending on the stimuli and on the psychophysiological starting-position of the organism. Enhancement occurs not only in response to stimuli which most subjects rate as predominantly "unpleasant" but also when the self-ratings indicate predominantly pleasant" emotional reactions in most of the subjects, as in the case of viewing the comedy "Charley's Aunt". We interpret these data as supporting the hypothesis concerning stress (Selye)" as the non-specificity (or stereotypy) of physiological reaction to a *variety* of stimuli and the hypotheses presented in figures 1._ and 1.3, taking into account not only unpleasant reactions but "pleasant" ones as well. Probably it is the intensity and not the quality of these reactions which is the main correlate of stress (Selye). Our results further support the assumption that sympathoadrenomedullary activity constitutes part of stress (Selye) and agree with the findings recently reported by Pátkai (1971).

Of course, this is *not* meant to imply that no specific relationships exist between psychosocial stimuli and physiological response, or between subjective response and physiological concomitants. On the other hand, our findings do not support hypotheses proposed by other authors concerning a specific relationship between e.g. anxiety and adrenaline excretion, or between aggression and noradrenaline excretion. This interpretation is in agreement with findings reported by Frankenhaeuser and her group (for review see Frankenhaeuser 1971).

True, the stress (Selye) non-specificity in physiological response discussed so far relates exclusively to *psychosocial* stimuli. On the other hand, it is well known that a considerable number of *physical* environmental stimuli do evoke a similar response, inter alia involving sympathoadrenomedullary activity. In a recent study conducted in collaboration with our laboratory a group of young, healthy male volunteers were exposed either to low environmental temperature (not combined with any psychosocial stressors) or to a sequence of psychosocial stressors (not combined with any uncomfortable climatic conditions). Both exposures lasted 24 hours and were preceded by 24-hour control periods. It was found (Lennquist, 1972 Lennquist et al., 1972) that both exposures elicited almost identical sympathoadrenomedullary and renal reactions.

Against the background of these results one may ask (cf. Mason, 1971) whether this stereotypy reflects a genetically determined psychobiological program (box 2, cf. figure 1.1) in Selye's sense, or whether the common denominator in both cases was the *experienced unpleasantness* of the exposures, the physiological concomitants of which we have registered. To check this possibility an attempt was made in collaboration with Ove Wilson and his group to expose subjects to cold without their becoming aware of it (Wilson et al., in preparation) namely during sleep. This was accomplished by discretely removing the framed blankets (shaped like half cylinders) while the subjects were asleep in a climatic chamber (at 10 and 20 C) without wakening them. Urine samples were collected but, unfortunately lost in transport. The study is mentioned here for methodological purposes only.

As indicated in paragraph 1.8 there can be several *degrees* of non-specificity in bodily response, the same reactions occurring in response to (a) a relatively great diversity of *situations* (b) a relatively great diversity of *stimuli* (physical and/or psychosocial), or (c) *every* stimulus. Our results do not allow any definite statement as to which of these alternatives is most valid, but they do contribute to illustrate the non-specificity postulated by Selye and emphasize the need for further research in this field.

8.4 The physiological significance of changes in free urinary catecholamines

As indicated in paragraph 2.14 the interpretation of changes in urinary catecholamine excre

tion is by no means simple (cf. Sapira and Bron, 1971). As emphasized by Luce (1969), "nobody imagines that brain events are precisely measurable in urine. Neither are sympathoadrenomedullary or say cardiovascular events. On the other hand, the same author points out that enough information can be inferred from urine analysis to make it a kind of chemical EEG".

Mason (1968) quite correctly asks whether changes in urinary output of epinephrine and norepinephrine might under some conditions reflect changes in the metabolism or percentage of excretion rather than the rate of internal secretion of these compounds". Thus the urinary excretion of *free catecholamines does probably not provide* a quantitative but a *semiquantitative* measure of general sympathoadrenomedullary activity. Plasma catecholamine analyses and analyses of catecholamine metabolites in urine in man (for a review see O'Hanlon, 1970) and determinations of relevant enzymes and tissue catecholamines in animals (cf. Rubenson, 1969; Kvetňansky et al., 1970; Axelrod et al., 1970), indicate that a variety of stressor exposures do, indeed, evoke an enhanced formation and/or release and/or turnover of adrenaline and noradrenaline. It can therefore be safely assumed that if there is an increase in adrenaline *excretion*, it is mostly preceded by an increase in sympathoadrenomedullary activity in the organism. If the excretion reaches *high* levels, sympathoadrenomedullary activity is probably high. It is conceivable that such a sympathotonia, if prolonged, can be of pathogenic significance (cf. Raab, 1971; Hermann and Mornex, 1964).

8.5 Catecholamine excretion as a predictor of subjective reactions

The methods for measuring subjective response and sympathoadrenomedullary activity are both, no doubt, relatively crude. In spite of this, highly significant correlations have been found between these two sets of variables. As shown in Chapter 7, the correlations could reflect circadian co-variation and/or co-variation in these responses as evoked by various stressors. The relationship is no doubt complex and probably influenced to a certain degree by stimulus as well as by response specificity. Even so, significant correlations have been found between subjective and physiological variables, and between these variables and performance, complementing the findings reported by Frankenhaeuser and her group (for a review see Frankenhaeuser 1971).

8.6 Some implications for evaluation of laboratory data in clinical practice

With reference, inter alia, to data presented in Chapter 7, The Lancet emphasizes in a leading article (May 20, 1967, pp. 1091) that environmental factors must be taken into account *when evaluating laboratory data in clinical practice*.

Such factors may be particularly confusing in studies where the physician almost exclusively relies on laboratory data, as in mass screening by laboratory investigations of large populations. But even in general practice and in internal medicine there seems to be a growing tendency to rely on laboratory data alone, at the expense of a thorough anamnesis and a clinical status.

A patient may, for instance, complain of fatigue, and turn out to have clearly subnormal levels of serum iron. Or he may exhibit protein-bound iodine levels that are clearly above normal, his main complaints being nervousness, anxiety and distress. But which is the hen and which is the egg? According to our hypotesis, *both* are "eggs", the "hen" being the interaction between the exposure to e.g. psychosocial stressors and the individual's psychobiological program. At all events, there seems to be good reason to inquire about sleeplessness and distress, about life changes, frustrations and social conflicts in patients exhibiting e.g. a catecholamine excretion level close to what is usually considered as indicative of pheochromocytoma, increased plasma lipids, depressed ST and T in the ECG, high PBI or low serum iron levels.

Briefly, then, the patient's psychosocial situation can influence rather markedly the various measures obtained at a department for clinical chemistry or clinical physiology. Failure to realize this may lead to serious diagnostic errors.

8 7 Some clinical and research implications

Although present knowledge does not allow any definite conclusions as to the *pathogenic* significance of the physiological reactions demonstrated in Chapters 1—7 it may be tempting to speculate somewhat on this issue.

As emphasized in our review in Chapter 1 and in paragraph 8.—, several authors have tried to identify psychosocial stimuli that might be of pathogenic significance, as well as high risk groups that have an increased propensity to react to such stimuli by disease. In general, attempts have been made to describe the contents of the various "boxes" shown in figure 1 1 (p. 12) and to relate them to each other So far most of the evidence available is of an associative nature, and no serious attempts ha e been made to probe the entire pattern in man, probably because of the difficulty in assessing this pattern in a suit able study design.

Kagan and Levi (197?) have recently proposed that at the present stage the following hypotheses are ripe for testing:

(a) *Contr l of psychosocial environment* (box 1, figure 1 1) *educes diseas*

(b) *Cont ol t psychological and/or physiological reaction* b x 3 figure 1 1) *reduces disease.*

(c) These responses are *interrelated* and are me diated thr u h *neuroendocrine mechanisms* as a final com on pathway

This approach might eventually reveal the key t many problems of prevention and treatment" sought by Flanders Dunbar (cf. paragraph 1 7 1)

One research strategy would comprise phar macological intervention (cf Leanderson and Levi. 1966) In addition, and probably no less important, part of this research might be conducted as an *integral part of social action programs* decided upon by health administrators, by introducing multidisciplinary *evaluation* of the effects of the social policy measures, i.e. of controlled intervention. By studying in the same setting the stimuli, the various characteristics of populations exposed (or not exposed) to these stimuli, their physiological and psychological eactions over time in longitudinal, multidisciplinary studies, and finally the outcome in terms of health, disease, psychological, social and economic function, we may eventually be able to provide decision makers with at least some of the relevant information for their political action (Kagan and Levi, 1972)

To be able to do this we must refine our me thodology for *assessing* psychosocial stimuli, psy chological and physiological reactions, precursors of disease, and disease, in different cultures.

No one would deny the great difficulties involved, nor the great need for this type of large scale multidisciplinary research programs.

So far very little has been said about *interacting variables* (cf. box 6 figure 1 1) In the present context we would like to mention just a few namely tobacco, alcohol, and caffeine-containing beverages. As mentioned above, psychosocial stimuli have been demonstrated to influence sympathoadrenomedullary activity and lipid me tabolism in a direction that might, under certain circumstances, become disease-provoking. As shown in studies conducted at our laboratory (Levi, 1967 Fröberg et al., 1969· Brohult et al., 1970) and by other investigators (e.g. Frankenhaeuser et al., 1968, 1970) these every-day stimulants" seem to induce very similar reactions. Epidemiological studies have furnished some additional evidence of tobacco, alcohol and caffeine abuse as possible risk factors. Accordingly ef fects on health and disease of these extremely widespread pharmacological influences and their possible *interaction* with the effects of psychosocial stimuli should be studied. More research should also be devoted to the corresponding influences of supposedly beneficial interacting variables such as physical activity (cf. Raab, 1966) and a balanced, adequate nutrition.

A few words should also be said concerning possible implications of the circadian rhythms demonstrated in Chapter 7 In internal medicine and psychiatry we may be on look-out for biochemical correlates of the exacerbations and re missions of various pathological states, possibly finding a key to their etiology and pathogenesis. In addition, the marked fluctuation and covariation, with or without time lag, in psychological performance and physiological variables and the

sequence between their respective crests offer an opportunity to approach several interesting psychophysiological relationships. Some of the rhythms may be pharmacologically modifiable. Some may offer important information as to how to plan man's various procedures in shift work, or with regard to continuous, long term duty

8.8 References

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9 SUMMARIES

By Lennart Levi

9.1 Summary

Using Selye's physiological stress concept as a starting point, the methodologic prerequisites for a scientific study of the influence of psychosocial stimuli on psychological and physiological reactions in the human organism are described. A number of experimental studies are reported, focused on reactions assumed to be relevant for psychiatry and internal medicine. The studies comprise a number of variables, the measurement of urinary catecholamines as proposed by Euler being focused upon.

It is well known that physical stimuli can evoke disease. This has been demonstrated for a considerable number of stimuli and diseases. The relationship between psychosocial stimuli and disease is less clear. In Chapter 1 the key terms are defined and a theoretical model is presented for the possible pathogenic effects of such stimuli.

A number of studies are reviewed elucidating the relationship between psychosocial stimuli and physiological mechanisms, between these mechanisms and various diseases, and between the stimuli and the diseases. The mechanisms focused on are primarily sympathoadrenomedullary, adrenocortical and thyroidal.

The chapter concludes by presenting the primary aims of this monograph: (a) mapping out the influence of psychosocial stimuli on various physiological mechanisms, (b) study of the relationship, if any, between experimentally induced psychological and physiological reactions, (c) comparison between physiological reactions to short term and long term stressor exposures, and (d) discussion concerning the relationship, if any, between the physiological reactions thus evoked and the pathogenesis of various diseases.

Chapter 2 comprises a detailed presentation of various sources of error and various techniques relevant for psychophysiological research. Some *guide lines for the optimal design of such studies* and the methodology of the studies comprised in the following chapters are presented.

Chapter 3 presents a study based on Selye's hypothesis that sympathoadrenomedullary and related reactions comprised in the "stress (Selye)" concept can occur as concomitants not only of psychological reactions usually rated as "unpleasant" but of "pleasant" reactions as well. In contrast, in situations evoking indifference, the level of "stress (Selye)" as reflected e.g. in adrenaline excretion, should be low. To test this hypothesis, 20 young female office clerks, acting as their own controls, were presented with a different 1 1/2 hour film on each of several consecutive evenings. It was found that the calmness and equanimity induced by viewing bland natural-scenery films was reflected on a biochemical level by a significant lowering of the catecholamine excretion. In contrast, the agitating and aggression-provoking "Paths of Glory", the anxiety provoking "The Mask of Satan" and the amusing comedy "Charley's Aunt" all induced significant increases in adrenaline excretion. These results support the non-specificity in physiological reactions postulated by Selye.

In Chapter 4 a similar study is reported, against the background of the Kinsey hypothesis that males are more prone than females to sexual arousal from visual stimuli. Accordingly, a total of 53 female and 50 male students were shown a 1 1/2-hour film program, comprising four short, silent films chosen to induce predominantly pleasant sexual arousal. Adrenaline and noradrenaline excretion increased significantly in both groups during the film period in relation to control levels before and after. During the film period, sexual arousal was the predominant sub-

jective reaction reported by both sexes, the self rating scores as well as their increases, however being significantly higher in the male group. This difference in reported subjective reactions was paralleled by a corresponding difference in the urinary excretion of adrenaline. These results are interpreted to support the "stress (Selye)" concept as well as the Kinsey hypothesis mentioned above. In this study, as well as in the previous one, significant changes occurred in urine flow, specific gravity and creatinine excretion.

The background of the study presented in Chapter 5 was a hypothesis concerning pathogenic consequences of a prolonged enhancement of sympathoadrenomedullary activity, e.g. for the cardiovascular system. It is often assumed that this may be due to cardiotoxic effects of the catecholamines per se but also to their lipolytic effects, eliciting an increased release of free fatty acids from the adipose tissue and, eventually, a hyperlipoproteinemia.

To test this hypothesis, 11 middle-aged males were exposed to a simulated industrial situation involving sorting ball-bearings for 2 hours to the accompaniment of distracting noise and lights. This exposure was found to evoke distress reactions of moderate intensity, accompanied by increases in heart rate, systolic blood pressure and catecholamine excretion but also of free fatty acids and triglycerides in arterial plasma. No such reactions occurred in a control group not exposed to this situation. The stressor-induced *increases in free fatty acids and triglycerides but* not in cardiovascular or sympathoadrenomedullary reactions were significantly modified by the administration of an antilipolytic drug, nicotinic acid. The results support our hypothesis concerning the genesis of hyperlipoproteinemia in response to psychosocial stimuli. It has been hypothesized that such a hyperlipoproteinemia may, in turn, be significantly related to atherosclerosis and degenerative heart disease, but possibly also to morbidity and mortality in general.

It is often claimed that psychosocial stimuli inherent in real life provoke disease. Very often, this assumption is made in relation to psychosocial aspects of working life. Should this be so, one would expect that conditions of work should be able to evoke reasonably pronounced reactions of the "stress (Selye)" type, primarily enhanced sympathoadrenomedullary activity.

In Chapter 6, the study concerned 12 healthy female invoicing clerks facing conditions very similar to those involved in their every-day work, a number of extraneous physical and psychosocial stimuli, however, being kept under control. Highly progressive piece wages were introduced on the first and third day of the experiment, and were found to result in significant increases in output but also in rush, fatigue and physical discomfort ratings, in adrenaline, noradrenaline and creatinine excretions and in urine flow. Accordingly every-day conditions at work can significantly modify physiological reactions in a way that *might* be of pathogenic significance for the human organism.

Chapter 7 reports sympathoadrenomedullary reactions in response to a distress- and fatigue-provoking situation lasting 3 days and nights, to which 31 young and middle-aged Army officers and corporals were exposed. It was found that the exposure was accompanied by significant increases in adrenaline excretion, and in protein-bound iodine in plasma, in individual cases to levels clearly above the normal range. Serum iron levels decreased dramatically, reaching subnormal levels in all subjects but three. Significant circadian rhythms were found in sympathoadrenomedullary, renal, performance and self rated *variables and significant psychophysiological cor*relations are described.

Finally Chapter 8 discusses existing evidence supporting the hypothesis (figure 1.1, page 12) that psychosocial stimuli can, indeed, evoke disease. The author further discusses and supports Selye's stress concept, and the concept of catecholamine excretion as a correlate of subjective reactions. It is emphasized that the patient's psychosocial situation must be taken into consideration when data from clinical laboratories are evaluated. The author draws attention *to the need* to focus not only on studies presenting evidence of an associative nature but, in addition, on the testing of hypotheses that control of the psy-

chosocial environment and/or of man's psychophysiological reactions *reduces disease* and that these responses are interrelated and are mediated through neuroendocrine mechanisms as a final common pathway. Finally, emphasis is placed on the need for studies also taking into account predisposing or protective interacting variables such as every-day stimulants, physical training and a balanced nutrition.

9.2 Zusammenfassung: Stress und Unlust als Reaktionen auf psychosoziale Stimuli. Labor- und Feldstudien betreffend sympathoadrenomedullare und verwandte Reaktionen

Der Autor hat einen theoretischen Referenzenrahmen von dem physiologischen Stressbegriff Selye's ausgehend angegeben und die methodologischen Voraussetzungen eines wissenschaftlichen Studiums der Einwirkung psychosozialer Stimuli auf die psychologischen und physiologischen Reaktionen des menschlichen Organismus beschrieben. Mehrere experimentelle Studien werden präsentiert, worin solche Reaktionen studiert worden sind, von welchen vermutet wird für Psychiatrie und Innere Medizin von Relevanz zu [illegible] von Ulf von Euler vorgeschlagenen [illegible]ssungen von Katecholaminen im Urin.

Dass physikalische Stimuli Krankheit hervorrufen können, ist wohl bekannt, was eine bedeutende Anzahl Stimuli und Krankheiten betrifft. Bezüglich psychosozialer Stimuli ist der Zusammenhang bedeutend schlechter klargelegt.

Im Kapitel 1 definiert der Autor seine Fachausdrücke und präsentiert ein theoretisches Modell, wie solche Stimuli Krankheit verursachen können. Er berichtet über eine Anzahl Untersuchungen, die den Zusammenhang zwischen solchen Stimuli und physiologischen Reaktionen, zwischen den Reaktionen und verschiedenen Krankheitszuständen, und zwischen Stimuli und den Krankheitszuständen beleuchten. Das Kapitel wird mit Angabe der Hauptzwecke der Abhandlung beendet:

(a) Klarlegung der Einwirkung der psychosozialen Stimuli auf verschiedene Körperfunktionen, (b) Zusammenhang zwischen experimentell hervorgerufenen psychischen und physiologischen Reaktionen, (c) Vergleich der Reaktionen auf kurze und langwierige psychosoziale Stimuli und (d) Diskussion des eventuellen Zusammenhanges der entstandenen Reaktionen mit der Pathogenese verschiedener Krankheiten.

Im Kapitel 2 macht der Autor eine detaillierte Durchnahme der verschiedenen Fehlerquellen und *Techniken mit Relevanz für psychophysiologische* Forschung. Er gibt einige Richtlinien an, für Anlage solcher Studien und berichtet zuletzt über das allgemeine Design und die Methodik der Studien, die in der Abhandlung enthalten sind.

Im dritten Kapitel geht der Autor von der Hypothese aus, dass sympathoadrenomedullare und andere Reaktionen, die zum Stressbegriff Selye's gehören, als Begleitphänomene nicht nur unangenehmer, sondern auch angenehmer psychischen Reaktionen entstehen können. Er beschreibt ein Experiment, in welchem man [illegible]0 Versuchspersonen während vier nacheinander folgenden Abenden unter streng standardisierten Bedingungen vier verschiedene Filme, unter Registrierung psychischer und sympathoadrenomedullarer Reaktionen, gezeigt hat. Der neutrale Kontrollfilm resultierte in einer Senkung der Katecholaminenausscheidung mit dem Urin, während der dramatische, komische bzw. schreckerregende Film trotz offensichtlichen Verschiedenheiten in den selbstbewerteten psychologischen Reaktionen der Individuen durchgehend eine Zunahme der Adrenalinausscheidung und des Urinvolumens und eine Abnahme des spezifischen Gewichts des Urins hervorrief. Dies deutet auf die von Selye postulierte Reaktionsstereotypie hin.

Im Kapitel 4 prüft der Autor mit gleichartiger Methodik noch einen Typ von Stimuli, nämlich Filme mit erotischem Inhalt. Er vergleicht dabei die Reaktionen weiblicher und männlicher Versuchspersonen und findet heraus, teils dass Versuchspersonen beider Geschlechter, obwohl die Filme hauptsächlich als angenehm empfunden wurden, mit bedeutenden Zunahmen in der Katecholaminausscheidung mit dem Urin reagieren,

teils dass die männlichen Versuchspersonen, als Gruppe betrachtet, stärkere sowohl psychologische als physiologische Reaktionen auf die Filmvorführung aufweisen, in Übereinstimmung mit der Hypothese Kinsey's darüber dass Männer in der Regel stärkere Reaktionsneigung auf visuelle sexuelle Stimuli haben.

Im fünften Kapitel werden in einer simulierten Arbeitssituation die Mechanismen hinter der Hyperlipoproteinemie studiert, die der Reaktion auf verschiedene Arten psychischer Belastungen zugeschrieben worden ist. Die Studie zeigt, dass die Stimuli, die verwendet worden sind, sowohl psychische Unlustreaktionen wie Blutdruck und Pulssteigerungen, erhöhte Ausscheidung mit dem Urin von Adrenalin und Noradrenalin und Steigerungen freier Fettsäuren und Triglyceriden im arteriellen Plasma haben hervorrufen können.

Die letztgenannten Reaktionen konnten durch Behandlung mit Nikotinsäure stark modifiziert werden. Die Resultate sprechen dafür dass die von den psychosozialen Stimuli hervorgerufene Sympathotonie zu einer Mobilisierung freier Fettsäuren aus den Fettdepots und sekundär zu einer Hyperlipoproteinemie führt. Die beiden letztgenannten Glieder in der Ereigniskette konnten ganz oder teilweise mit der antilipolytischen Nikotinsäurenbehandlung blockiert werden.

Im Kapitel 6 ist als Stimulus ein Leistungslohn verwendet und die Studie in einer wirklichen Arbeitssituation durchgeführt worden. Das Lohnsystem der Versuchspersonen wurde experimentell von Zeit auf Leistungslohn geändert, und die Einwirkung auf Leistungen, selbstbewertete Erlebnisse und sympathoadrenomedulläre Reaktionen wurden studiert. Die Einführung eines hohen und progressiven Leistungslohnes wurde von signifikanten Reaktionen sämtlicher studierten Variablen begleitet. Die prinzipielle Applizierbarkeit der Methodik für Studien psychophysiologischer Effekte verschiedener Umstände im Arbeitsleben und in anderen Milieus wurde betont.

Kapitel 7 berichtet über psychologische und physiologische Reaktionen auf Exposition eines 3×24 Stunden andauernden Wachhaltens unter anstrengenden äusseren Verhältnissen. Der Autor zeigt, dass die in früheren Kapiteln beschriebenen sympathoadrenomedullären Reaktionen auch hier vorhanden sind und sogar verstärkt werden, wenn die Exposition langwierig gemacht wird. Ferner werden Daten präsentiert, die zeigen, dass das proteingebundene Jod in vielen Fällen steigt und dass das Serumeisen in der Regel auf Werte sinkt, die über respektive unter den Normalvariationen für diese Variablen liegen. Die statistischen Zusammenhänge zwischen psychischen und physiologischen Reaktionen und deren Abhängigkeit von (a) der Dauer der Belastungen und (b) deren zirkadianen Rhythmus werden präsentiert und diskutiert.

Das achte Kapitel, schliesslich, diskutiert den Zusammenhang zwischen psychosozialen Stimuli, physiologischen Reaktionen auf diese Stimuli und Pathogenesen verschiedener Krankheiten. Ferner wird der Stressbegriff Selye's diskutiert sowie die Katecholaminausscheidung mit dem Urin als Korrelat subjektiver Reaktionen. Weiter wird hervorgehoben, dass die psychosoziale Situation des Patienten bei der Auswertung von Labordaten in Betracht gezogen werden muss, dass Anhaltspunkte vorhanden sind, dass gewisse Situationen krankheitsverursachend zu sein scheinen, und dass es nun angezeigt ist, die Hypothesen zu testen, dass therapeutische Eingriffe in die psychosoziale Situation und/oder in die subjektive und physiologische Reaktionen des Individuums Krankheit verhindern können. Zuletzt wird auf die mögliche krankheitsverursachende und krankheitsverhindernde Bedeutung interagierender Variablen hingewiesen wie Tabak, Alkohol und Kaffee als Beispiel der erstgenannten, physisches Training und eine balancierte Ernährung als Beispiel der letztgenannten.

9 3 Resumé Stress et sentiments de malaise en tant que reactions aux stimuli psycho-sociaux. Études pratiques et de laboratoire des réactions sympatho adrenomé dullaires et apparentées

Se basant théoriquement sur la notion de stress physiologique de Selye, l auteur décrit les conditions méthodologiques nécessaires à l étude scientifique de l'influence des stimuli psycho-sociaux sur les réactions psychologiques et phy siologiques de l organisme humain. Il rend compte de nombreuses études expérimentales de tels types de réactions, semblant bien être du ressort de la psychiatrie et de la médecine interne, notamment à l'aide des mesures de catécholamines dans l urine (méthode Ulf von Euler)

Il est bien connu que des stimuli physiques peuvent provoquer des maladies. On en a la preuve pour un nombre important de stimuli et de maladies. En ce qui concerne les stimuli psycho-sociaux, la relation est notablement moins clairement établie

Dans le premier ch p tre, l auteur définit les termes qu il emploie et présente un schéma théorique des effets pathogeniques possibles des stimuli psycho-sociaux.

Il cite diverses études prouvant la relation entre des stimuli de ce type et certaines réactions physiques, entre ces eactions et divers états pathologiques, et entre ces stimuli et les maladies. Les réactions spécialement étudiées sont sympatho-adrénomédullaires, adréno-corticales et thyroïdiennes.

Le chapitre s achève sur l indication des prin cipaux buts de la thèse (a) détermination de l'influence des stimuli psycho-sociaux sur diverses fonctions du corps, (b) étude des relat ons entre réactions psychiques et physiologiques artificiellement provoquées, à titre expérimental, (c) comparaison des réactions à des stimuli psycho-sociaux de brève et de longue durées, (d) discussion des relations éventuelles entre les réactions obtenues et la pathogénèse de diverses mal d es.

Dans le deuxième chapitre, l auteur passe en revue détaillée diverses sources d erreurs et diver ses techniques en rapport avec la recherche psycho-physiologique. Il indique les grandes lignes d'une étude bien menée dans ce domaine et pré sente le plan général et la méthode des études incluses dans les chapitres suivants.

Le chapitre trois présente une étude fondée sur l'hypothèse que les réactions sympatho-adréno-médullaires et autres embrassées par la notion de stress de Selye, peuvent non seulement accompagner des réactions psychiques ressenties comme désagréables, mais aussi des réactions éprouvées comme plaisantes. Inversement, dans des situations neutres » le niveau de stress (toujours selon Selye) tel que révélé par l'excrétion d'adré naline notamment, devrait être bas. Pour vérifier cette hypothèse, 20 jeunes employées de bureau ont été invitées quatre soirs de suite, dans des conditions strictement standardisées, à voir quatre films différents, au cours desquels étaient enregistrées les réactions psychiques et sympatho-adrénomédullaires. Le calme et la tranquillité d'esprit suscités par la projection d'un documen taire présentant de jolis et doux paysages se sont traduits au niveau biochimique par une baisse significative de l'excrétion de catécholamines. Par contre un film bouleversant et éveillant l'agressi vité tel que Les sentiers de la gloire angoissant tel que Le masque de Satan et une amusante comédie comme La tante de Charley ont tous trois provoqué une typique augmenta tion de l excrétion d'adrénaline, malgré les évidentes différences des réactions psychologiques ressenties par les participantes au test. Le volume des urines s est accru tandis que diminuait le poids spécifique de ces urines. Ces faits viennent à l appui du postulat de Selye sur la stéréotypie des réactions physiologiques.

Le chapitre quatre est consacré à une étude similaire, tenant compte de l'hypothèse de Kinsey selon laquelle les hommes sont plus sensibles que les femmes à une excitation sexuelle provoquée par des stimuli visuels. Quatre courts-métrages muets, choisis pour provoquer une excitation sexuelle, on été montrés à 53 étudiantes et 50 étudiants. En comparaison des niveaux de contrôle

nant et après la séance, l'excrétion d'adrénaline et de noradrénaline a augmenté de manière significative dans les deux groupes, durant la projection. Au cours de celle-ci, l'excitation sexuelle a été la réaction subjective prédominante constatée par les deux sexes, qui l'ont ressentie comme tout plaisante, mais le groupe masculin dans son ensemble a accusé des réactions tant psychologiques que physiologiques nettement plus fortes, se traduisant par une plus importante excrétion d'adrénaline que chez les femmes. Ces résultats confirment à la fois la notion de stress de Selye et l'hypothèse ci-dessus mentionnée de Kinsey. Comme dans l'expérience citée au chapitre trois, on a pu constater des modifications significatives du volume urinaire, du poids spécifique des urines et de l'excrétion de créatinine.

Dans le chapitre cinq, l'auteur étudie les mécanismes amenant l'hyperlipoprotéinémie qu'on pense provoquée par la réaction à diverses sortes d'épreuves psychiques. L'expérience simulait une situation dans le travail, et prouve que les stimuli utilisés étaient susceptibles de provoquer non seulement des réactions psychiques (sentiment désagréable) mais encore une hausse de la pression sanguine, une accélération du pouls, un accroissement de l'excrétion de l'adrénaline et de la noradrénaline avec l'urine, et une augmentation du taux des acides gras libres et des triglycérides dans le plasma. Un traitement à l'acide nicotinique a pu modifier fortement ces dernières réactions. Les résultats semblent démontrer que la sympathotonie provoquée par les stimuli psycho-sociaux mène à une mobilisation des acides gras libres des dépôts adipeux et, à titre secondaire, à une hyperlipoprotéinémie. Ces deux derniers maillons de la chaîne des réactions ont pu être entièrement ou partiellement bloqués par le traitement anti-lipolytique à l'acide nicotinique. On a lancé l'hypothèse que l'hyperlipoprotéinémie peut à son tour être mise en relation de cause à effet avec l'athérosclérose et la dégénérescence cardiaque, ainsi peut-être qu'avec la morbidité et la mortalité en général.

On a souvent affirmé que les stimuli psycho-sociaux inhérents à la vie réelle engendrent la maladie. Très souvent cette affirmation est faite en relation avec des aspects psycho-sociaux du travail. Si tel est bien le cas, on doit pouvoir s'attendre à ce que les conditions de travail suscitent des réactions de stress (au sens de Selye) en premier lieu une augmentation de l'activité sympatho-adrénomédullaire.

Alors que dans l'expérience précédente on avait soumis onze hommes d'âge moyen à une situation industrielle simulée (tri de roulements à billes pendant deux heures avec accompagnement de bruits et de lumières gênants) l'expérience relatée dans le chapitre six s'est déroulée dans une situation réelle, avec douze femmes en bonne santé, établissant des factures. Néanmoins un certain nombre de stimuli physiques et psycho-sociaux n'ayant rien à voir avec le problème ont été maintenus sous contrôle. Les premier et troisième jour de l'expérience, le traitement mensuel de ces femmes a été remplacé par un salaire à la pièce, stimulant la productivité. Il en est résulté une augmentation significative de la production mais aussi de la presse, de la fatigue, de l'inconfort physique, des taux d'adrénaline, noradrénaline et créatinine, et du volume urinaire. Par conséquent les conditions de travail quotidiennes sont manifestement susceptibles de modifier les réactions physiologiques humaines dans un sens pathogénique. La méthode s'avère capable d'une application pratique dans l'étude des effets psychophysiologiques des diverses conditions de travail.

Le chapitre sept rend compte des réactions psychologiques et physiques d'un groupe de trente et un officiers et sous-officiers, jeunes ou d'âge moyen, soumis durant trois journées de 24 heures, sans sommeil, à des conditions extérieures éprouvantes. L'auteur montre ici que les réactions décrites dans les chapitres précédents non seulement se révèlent mais encore sont renforcées par la longueur de l'épreuve. On retrouve l'augmentation significative de l'excrétion d'adrénaline et en outre on a pu noter dans plusieurs cas un accroissement de l'iodine liée aux protéines dans le plasma ainsi qu'une baisse en général de la teneur en fer du sérum, l'un et l'autre supérieurs aux variations normales de ces variables. La baisse de la teneur en fer a été spectaculaire sauf dans trois cas. Des rythmes circadiens n'ayant

ont été découverts dans l'activité sympatho-adrénomédullaire et rénale et dans les variables subjectifs et de performance. Les relations statistiques entre réactions psychiques et physiologiques et leur dépendance de (a) la durée des épreuves et *(b) leur rythme circadien sont présentées et discutées.*

Le huitième chapitre, enfin, discute les relations entre stimuli psycho-sociaux, réactions physiologiques à ceux-ci et la pathogénèse de diverses maladies. Les preuves existantes confirment l'hypothèse que les stimuli psycho-sociaux peuvent réellement engendrer des maladies. L'auteur discute et soutient, en outre, la notion de stress selon Selye, et le concept de l'excrétion de catécholamines avec l'urine, corrélative aux réactions subjectives. Il souligne le fait que la situation psycho-sociale du patient doit être prise en considération lors de l'appréciation des données de laboratoire clinique, qu'il existe des preuves que certaines situations peuvent engendrer des maladies, et qu'il est désormais important de tester les hypothèses selon lesquelles des interventions thérapeutiques dans la *situation psycho-sociale* peuvent contrer la maladie et des interventions dans les réactions subjectives et physiologiques de l'individu à l'exposition à une situation provoquant normalement la maladie peuvent neutraliser la maladie. Les mécanismes neuro-endocriniens relient ces *réponses*—corrélatives en tant que canal commun final. Pour terminer l'auteur souligne l'importance possible de l'interaction de certaines variables pouvant provoquer la maladie (tabac, café, alcool par exemple) ou au contraire la combattre (éducation physique, diète équilibrée, notamment)

ЛЕНАРТ ЛЕВИ

СТРЕСС И ИСТОЩЕНИЕ КАК О[illegible]

9.4 Заключение

Отправной позицией проведенных [illegible] ция стресса Г. Селье. В моног[illegible] методических приемов используе[illegible] циальных влияний в возникновении [illegible] ловека. Излагаются результаты экс[illegible] различных патологических реакций [illegible] к соматической клиниках. Работа с[illegible] полученных в результате исследований [illegible] в моче по методу Эйлера и Лишайко

Известно, что различные физические стим[illegible] возникновению тех или иных заболеваний. [illegible] далеко еще не ясным характер связей меж[illegible] ляниями и различными соматическими забо[illegible] ей монографии определяется исходная поз[illegible] оретическая модель предполагаемых патогенет[illegible] возникающих в результате такой стимуляции [illegible] результаты исследований, которые позволя[illegible] ления связи между указанными стимуляциями, [illegible] их влиянием патогенетическими механизмами и [illegible] ческих состояний. В процессе работы внимание [illegible] очередь было обращено на изучение механизмов [illegible] патоадреналовой, адренокортикальной и тиреои[illegible] заключение излагаются основные задачи работы: а/ Изучение эффектов возникающих под влиянием [illegible] стимуляции на различные физиологические механизмы, [illegible] вание связей между экспериментально вызванными псих[illegible] и физиологическими реакциями; в/ Установление различ[illegible] физиологическими реакциями под влиянием коротко действ[illegible] длительно действующих стрессовых воздействий; г/ Обсуждение предполагаемых связей между вызванными таким образом [illegible] гическими реакциями и патогенезом ряда заболеваний. Часть вторая посвящена детальному изложению технических пр[illegible] емов используемых для психофизиологических исследований и [illegible] суждению различных источников ошибок возникающих в процессе этих экспериментов. Приводятся некоторые оптимальные варианты таких исследований, а также основные методики применявшиеся в процессе данной работы.

Третья часть монографии включает в себя исследования основывающиеся на положении Селье о том, что симпатоадреномедуллярные реакции и другие проявления стресса могут возникать не

только как результат " неприятных " воздействий, но обнаруживаются также при " приятных " влияниях В противоположность этому индифферентная стимуляция не вызывает значительного изменения уровня проявления стресса В целях проверки этой гипотезы 20 молодым женщинам были ежедневно продемонстрировано несколько 1,5 часовых фильмов имеющих различное содержание Было установлено что видовые фильмы вызывающие чувство успокоения и расслабления, приводили к значительному снижению экскреции катехоламинов Наоборот фильмы вызывающие состояния напряжения страха и агрессии, а также комедийные фильмы вызывали выраженное повышение экскреции адреналина Эти данные подтверждают неспецифичность реакций описанных Селье

В четвертой части даны результаты экспериментов направленных на выяснение достоверности гипотезы Кинси, согласно которой мужчины имеют более высокий чем женщины уровень сексуальной возбудимости к зрительным стимулам В соответствии с этим, было исследовано 53 женщины и 50 мужчин / студенты / которым были показаны 1 1/2 часовые фильмы выбранные таким образом, чтобы их содержание могло вызвать сексуальное возбуждение Выявилось, что в период демонстрации этих фильмов экскреция адреналина и норадреналина достоверно повышалась в обеих группах по сравнению с контрольными определениями до и после киносеансов Во время просмотра фильмов согласно данным специально ориентированных психологических исследований сексуальное возбуждение доминировало в обеих группах Однако у мужчин оно было выражено в значительно большей степени Эти различия, выявившиеся при психологической оценке коррелировали с данными полученными при исследовании экскреции адреналина Результаты интерпретировались в поддержку концепции стресса Селье и гипотезы Кинси В процессе этой части исследования, также как и в предыдущих, наблюдались выраженные изменения в выделении ряда веществ с мочей ее удельном весе экскреции креатинина

Во главе угла исследований, которым посвящена пятая глава находилось положение согласно которому длительное напряжение симпатоадреномедуллярной системы является важным патогенным фактором особенно для сердечно-сосудистой системы. Предполагается что это может быть вызвано собственно кардиотоксическим эффектом катехоламинов и их липолитическим эффектом выражающимся в увеличении освобождения свободных жирных кислот и липопротеинемии С целью проверки этой гипотезы 11 мужчин среднего возраста были подвергнуты влиянию обычных для них условий работы, которая, однако проводилась в сопровождении отвлекающего шума и вспышек света Было установлено, что данное воздействие привело к возникновению определенных реакций выражавшихся увеличением числа сердечных сокращений повышением систолического давления усилением экскреции катехоламинов и по-

нижением содержания в плазме свободных жирных кислот и триглицеридов В контрольной группе, не подвергавшейся воздействию указанной ситуации, подобных реакций не наблюдалось Применение антилиполитических веществ / никотиновая кислота / могло в значительной мере ослабить сердечно-сосудистую и симпатоадреномедуллярную реакции Полученные результаты подкрепляют гипотезу касающуюся генеза гиперлипопротеинемии в ответ на психосоциальные стимулы Высказывается предположение, что такая липопротеинемия может находиться в этиопатогенетической связи с атеросклерозом и дегенеративными поражениями миокарда. Возможно также она оказывает влияние на общий процент заболеваемости и смертности населения в целом

Принято считать, что различные психосоциальные воздействия, являющиеся выражением обычных условий жизни провоцируют возникновение тех или иных заболеваний Очень часто такое предположение высказывается в связи с психосоциальными аспектами трудовой деятельности В таком случае следует согласиться, что в зависимости от характера и условий работы могут развиваться те или иные проявления стресса и в первую очередь повышение симпатоадрреномедуллярной активности

В шестой части монографии приводятся результаты исследований 12 здоровых женщин / служащих / в условиях очень схожих с их обычной трудовой ситуацией Одновременно они подвергались некоторым внешним воздействиям: прогрессивно-сдельная форма оплаты в первый и третий день эксперимента В результате было установлено значительное повышение чувства усталости физического дискомфорта / регистрация велась по соответствующим шкалам / Кроме того обнаружилось повышение экскреции адреналина, норадреналина и креатинина в моче Таким образом повседневные условия трудовой деятельности могут в значительной мере модифицировать физиологические реакции у человека, что в свою очередь может иметь патогенное значение

В седьмой части работы приводятся данные исследований симпатоадреномедуллярных реакций возникающих в ответ на ситуации, которые приводят к выраженному утомлению и истощению Изучение проводилось на 31 молодого или среднего возраста военнослужащих которые в течение трех суток были лишены сна Обнаружилось что эти условия сопровождались выраженным повышением экскреции адреналина и концентрации связанного с белком иода в плазме В некоторых случаях уровень этих веществ значительно превышал пределы нормы. Содержание железа в плазме резко снижалось достигая субнормального уровня, у 28 из 31 испытуемых Кроме того выявился выраженный циркадный ритм в симпатоадреномедуллярной почечной и других системах Были установлены некоторые психофизиологические корреляции

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Supplementum 529

Pulse-conducted Triangular Exercise-ECG Test

A feed-back system regulating work during exercise

By Matti Arstila

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

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PULSE CONDUCTED TRIANGULAR EXERCISE-ECG TEST

Dedicated to my father
K. E. Arstila, M.D

From the Department of Medicine (Head Professor Pekka Brummer M.D.), University Hospital, and the Department of Physiology (Head Professor Kaarlo Hartiala, M.D.), University of Turku, Finland

PULSE-CONDUCTED TRIANGULAR EXERCISE-ECG TEST

A FEED-BACK SYSTEM REGULATING WORK DURING EXERCISE

by

MATTI ARSTILA

TURKU 1972

Vammala 1972, Vammalan Kirjapaino Oy

CONTENTS

ABBREVIATIONS DEFINITIONS AND UNITS

BH	body height	cm
BW	body weight	kg
BSA	body surface area	m^2
HA	heart shadow area	cm^2
HV	heart volume	cm^3
PFR	peak flow rate	$l\ min^{-1}$
VC	vital capacity	l
BP	arterial blood pressure	mmHg
HR	heart rate	bts/min
PER	perceived exertion rating	units
T	time	min
wT	working time	min
W	work	kpm
TW	total work	kpm
TWI	total wok index = TW per kg body weight	$kpm\ kg^{-1}$
WL = P	work load = power	$kpm\ min^{-1}$
WLI	work load index = WL per kg body weight	$kpm\ min^{-1}\ kg^{-1}$
MVO_2	myocardial oxygen consumption	$ml\ min^{-1}$
$Max.VO_2$	maximal oxygen uptake (measured)	$l\ min^{-1}$
$Pr.max.VO_2$	maximal oxygen uptake (predicted)	$l\ min^{-1}$
AI	maximal oxygen uptake per kg body weight = Astrand's index	$ml\ min^{-1}\ kg^{-1}$

Other abbreviations

M	male(s)
F	female(s)
AP	angina pectoris patient(s)
MI	myocardial infarction patient(s)
CHD	coronary heart disease
X	exercise
PCT	pulse-rate conducted triangular (test)

I INTRODUCTION AND OBJECT OF THE STUDY

Importance of exercise electrocardiography

As the incidence of coronary heart disease (CHD) is continually increasing, the need to diagnose either the presence or absence of the disease also grows. Human as well as economic reasons dictate that all efforts to discover the disease in an individual should be made at such an early stage that, by means of combating measures, it is still possible to effect a continuation of reasonable health and of the ability to work.

The importance of electrocardiography in diagnosing CHD has been known for more than half a century already. Unfortunately the technically simplest method, i.e. the ECG recording of a resting subject, is not a sensitive enough test for discovering the disease at the initial stage, when the signs and symptoms appear only during exertion. An *ECG recording done during and after* exercise is notably better in this respect. The exercise-ECG is, however a technically difficult, time-consuming and sometimes even dangerous test, and for this reason its use has not become as extensive as it needs to be. In all it is obvious that the exercise-ECG test must be developed in such a way that it can be applied to every middle-aged person at a low cost, safely and with reliable results. *Many simple tests have been devel*oped with this problem in mind, but the simplicity of the methods has compensated for the reliability of the results. Particularly in epidemiological screening studies methods have been used by means of which it has been possible to prove the difference in prevalence of CHD in different groups of people. On the other hand, the question whether or not certain individuals are suffering from coronary disease cannot be reliably answered by these kinds of strictly standardized methods.

Requirements concerning test methods

In 1966 the author convinced that no existing test would be suitable for a reliable, safe and cheap examination of large groups of people, began to develop a new test starting from the following basic ideas

1. The technical difficulties must be overcome by technical means. The great initial cost of equipment is no real obstacle if automation can bring down the operating expenses. It is especially important that the ECG be recorded in relation to the exercise in such a way that a good interindividual comparability makes automatic analysis possible.
2. The reliability of the test results must, as far as the ECG is concerned, be as great as possible, the only limitation being the discrepancy which sometimes appears between myocardial ischemia and the recording of electro-physiological changes.
3. As an examination of large groups of people can only be carried out by a technical staff, the internal or inherent *safety of the test must be as good as* possible.
4. It must be possible to measure the amount of external work done during exercise for the following reasons.
   — It is important to show quantitatively how much work has been done when chest pain and/or ECG changes, or a

change in other parameters appear in a person.

— For a part of symptomless men this test for discovering heart disease is better motivated if given as a fitness test.

— The measuring of physical working capacity is in itself a good screening test, which, when giving a normal result, excludes any greater disturbances in the oxygen carrying system of the organism as a whole, and when giving a poor result, may promote the discovery of some latent disease.

A new test the PCT X test

In 1967 the author succeeded in principle in finding a type of test, which seemed to fulfill all the requirements made and described above. The new test, which was later called Pulse-conducted triangular exercise-ECG test in short the PCT X-(ECG)-test, was first introduced at the Second International Seminar of Ergometry in Berlin, 1967 where the procedure and in particular the methods were presented.

The central idea of the new test, from which all its characteristics are derived, is the way in which the work advances. This, in short, goes as follows: Firstly the subject works against a load, which is increased continuously from zero to the maximum possible, and secondly the progress is constantly regulated on the basis of the heart rate reaction induced by the exercise. The simplicity of the test and its flexibility i.e. its adaptability to the individual's needs, are achieved precisely because the subject's own organism controls the progress of the test. This way the exercise is equally hard, subjectively for everyone and a basis is created for a good comparability of the results. The feedback from the organism to the machine, a biotechnological principle, sets the new test apart from all previously presented tests of the same kind.

Aims of this study

In this study the test as a whole is described for the first time. In addition the results which have been obtained by using a non automatic hand-operated version are presented. Altogether the aim of the author is to give the following detailed data and information.

— Theoretical background of the PCT X test.
— Different possible ways of solving the technical details.
— Experiences and observations of the hand-operated version applied in practice.
— Ergometric normal values for different age groups and both sexes in certain population groups.
— Results from test series, in which the same person performed the new test several times, either unchanged or with a change in details, and also other ergometric tests, the results of which have been compared with those of the new one.
— Observations on applying the new test to patients suffering from coronary heart disease.

On the whole this paper will deal with *the problem of exercise in the exercise ECG test.* ECG problems will be discussed only briefly Attempts have also been made, by means of this study to decide whether it is worth while to continue developing the work to the next objective, i.e. the establishing and using of automatic test units working on the principle in question in extensive mass screening, in addition to the use in routine clinical ECG examinations.

II ROLE OF EXERCISE AND EXERCISE-ECG IN EXAMINATION OF CORONARY CIRCULATION

The characteristic symptom of coronary insufficiency retrosternal or left precordial pain, mostly accompanied with dyspnea, often appears at an early stage of the disease only during exercise. Exercise testing is useful in provoking symptoms in a situation where they can be evaluated. A quantitative or ergometric exercise test measures the amount of exercise which a person can still tolerate. This measurement gives some insight into the severity of the disease, even if the correlation with anatomic changes or the prognosis of the disease in any individual is only a rough one.

Perhaps even more important is the possibility in an exercise test, of observing some objective signs of the disease. These often precede the subjective symptoms and can appear even without chest pain. Electrocardiographic changes, especially depression of the ST segment and T changes, reflect electrophysiological and metabolic changes in the myocardium. Exercise electrocardiography is a simple means of measuring approximately the myocardial oxygen balance, i.e. the relationship between oxygen (or blood) supply and demand in the myocardium. If the ratio between supply and demand is below 1, the oxygen requirements of the heart are not fulfilled and the result is a state of hypoxia or ischemia in the myocardium. The purpose of the exercise is to upset the myocardial oxygen balance to such a degree that overt symptoms (angina pectoris or dyspnea) and/or signs (e.g. ischemic ECG changes) will develop in a person even with an atypical or latent disease. The severity of the ischemia depends on the intensity and the duration of the exercise. Work load, time and their relationship are essential determinants of the sensitivity and safety of the exercise-ECG test. The importance of these factors, load and time, have been somewhat negleted in the past. Most scientific workers in this field have mainly been interested in the electrocardiography It is the role of the exercise which will be more fully discussed in this paper

MYOCARDIAL OXYGEN CONSUMPTION DURING EXERCISE

The myocardial blood supply or coronary flow usually increases in relation to an increased demand. However in the case of a narrowed or stenotic coronary artery the augmentation of the flow is possible only to a critical level. Above this level myocardial hypoxia will develop. The local ischemia will work as a strong agent decreasing coronary vascular resistance, and further increasing the flow However if the balance still remains negative, overt manifestations of myocardial hypoxia will develop. In these circumstances other factors which will diminish the oxygen supply such as a drop of arterial (perfusion) blood pressure, anemia, or a lowered arterial oxygen saturation will unfavourably influence the balance. One of the determinants of the blood supply is the time available for coronary flow i.e. the duration of diastole. Any factor which will shorten the diastole or lengthen the relative duration of systole will potentially impair

the coronary circulation. In this respect tachycardia and aortic stenosis are notewor thy clinical conditions. Tachycardia induced by exercise disturbs the balance more than simple tachycardia because of the preload to the heart caused by increased venous return from the actively contracting muscles. On the other hand any factor that increases the myocardial oxygen *consumption* will potentially provoke myocardial hypoxia. Tachy cardia is one of the chief determinants of myocardial oxygen consumption. This will be reviewed and discussed below

Myocardial oxygen consumption (MVO_2) bears, according to Sarnoff et al. (1958) and Sarnoff and Braunwald (1959) little relation to the external work per se of the heart. These authors found in an isolated supported heart preparation that the chief determinant of O_2-consumption was the total tension developed by the myocardium. The events occuring during the contraction, the energetics of myocardium, and the conditions, especially the aortic pressure, were factors deter mining the total tension and oxygen requirement. A parameter called *tension t me index* (TTI), calculated as the product of the mean systolic pressure the duration of systole, and the heart rate was found to have much better correlation with MVO_2 than that of the left ventricular w rk, cardiac output, heart rate or the product f he rt rate x mean aortic, mean systolic or peak systoli aortic pressure. Neither was the filling pressure or flow of any prime importance, and thus ow also applied to the fiber length the left entricle However the rate of devel- pment of tension was not controlled in these periments In 1964 Monroe demonstrated that MVO_2 is largely associated with events occuring dur g the generation of pressure rather than w th the maintenance of entricular pressure. In 1965 Sonnenblick t al. pointed out discrepancies between the TTI and the MVO_2 which were seen when the velocity of contraction was augmented by sustained postextrasystolic potentiation produced by paired electrical stimulation o by the administration of norepinephrine or calcium infusion. These workers concluded that while the tension developed by the myocardium undoubtedly plays significant part in determining MVO_2, neither the pe k tensions nor the TTI can be the sol determinants, and they suggested that the elocity of myocardial f ber shortening is an important determinant Thus *velocity of contraction* is reflected in parameters which can be determined in an intact human heart such as the rate of rise of the left ventricular pressure (dp/dt), the rate of change of ventricular force (df/dt) measured with straingauge arches sutured to the ventricle t the time of cardiac operations or the rate of change of ventricular length measured with cineradiography from the distance between silver-tantalum markers. The maximum velocity of shortening at zero load, i.e. V_{max}, which is achieved by extrapolation of the force- elocity curve to zero load, is also a useful indicator of the contractile state of the myocardium. V_{max} can be calculated from high fidelity pressure measurements and left ventricular angiocardiograms (Hugenholtz et al. 1970, Sonnenblick et al. 1970). The value of the V_{max} technique has been challenged by Pollack (1971).

Braunwald (1969) states that myocardial tension development and the contractile state of the myocardium are the two major determinants of MVO_2 and that the basal oxygen cost, the cost of depolarization, the metabolic effect of agents such as catecholamines and the activation energy requiring ATP and calcium as well as the energy costs of maintenance of the active state are other minor determinants of the myocardial oxygen consumption.

Outside cardiac catherization laboratories simple means of studying and parameters like heart rate and blood pressure must largely be relied on. The heart rate is one of the most important determinants of the myocardial oxygen balance when there is a limitation to the increase of coronary blood flow The aortic blood pressure, especially the peak systolic pressure, is connected with the force of contraction and thus with the generation of tension in the wall of the left ventricle The product of heart rate and systolic aortic pressure, the *rate pressure product* was shown by Robinson (1967) to be consistently related to the onset of pain in angina pectoris patients on numerous occasions during various kinds of stress tests such as pedalling an ergometer in a sitting position, stepping up and down a Master's two-step apparatus, running up and down a flight of stairs, raising an arm while holding a weight, or solving arithmetical problems. The rate-pressure product was

corrected in few episodes in which the ejection time was altered by a factor observed/average ejection time. The ventricular size was supposed to have been without variations, and no correction according to the Laplace equation (wall tension = intraluminar pressure x radius) was done. Sowton et al. (1967) found a similar constant angina threshold for nearly every patient when angina was induced using atrial pacing in the supine position and the product of aortic ejection pressure, ejection time and heart rate (tension-time index by Sarnoff et al. 1958) was used as an index of myocardial oxygen consumption. Redwood et al. (1971) used the *triple product* of heart rate, systolic pressure, and ejection time when studying patients with angina during upright bicycle exercise. They found that for a given patient there was a stable relationship between the onset of angina and the triple product in repeated similar or modified tests.

LEFT HEART FAILURE DURING MYOCARDIAL ISCHEMIA

The effects of *tachycardia per se* on central hemodynamics and on MVO_2 can be studied using the technique of atrial pacing. Stein et al. (1966) and Braunwald et al. (1967) studied healthy men or patients with mild forms of cardiac disease. Sowton et al. (1967) Frick et al. (1968), Linhart et al. (1969) Parker et al. (1969 a, c) and Dwyer (1970) induced angina pectoris in CHD patients for hemodynamic studies. These investigations can be summarized by stating that normal persons and angina pectoris patients behave in a similar way if no pain or dyspnea is produced. However when the critical level of ischemia is reached hemodynamic alterations become abnormal.

Normally simple tachycardia has little influence on the cardiac output. An increase in heart rate produces reciprocal decline in stroke volume. Ventricular end-systolic and end-diastolic dimensions are reduced, the former more because the velocity of shortening and thus the stroke power of the ventricular muscle is augmented by tachycardia, perhaps because of an increase in the rate of interaction of the contractile sites (Podolsky 1962). The relative duration of systole is shortened providing a relatively longer diastolic filling period.

With an increasing heart rate in CHD patients, usually at the rate of 120—140 beats/min., myocardial ischemia will develop. The left ventricular filling pressure often rises, especially when pacing is continued in spite of anginal pain. After the cessation *of pacing the left ventricular end-diastolic* pressure does not return to normal when stroke work falls, but may continue to rise. This indicates that the ischemic ventricle operates on a depressed Frank-Starling ventricular function curve. This phenomenon has been interpreted as a reversible state of heart failure. Another explanation for this elevation in ventricular filling pressure is a reduction in diastolic compliance during transient ischemia. Regional abnormalities in ventricular contraction are common. Examples are akinesis or asynergy of multiple regions, each of which adds to the myocardial function defect. However there may be anginal pain and ECG changes even without any hemodynamic abnormalities when atrial pacing is used. This is in sharp contrast to the gross abnormalities observed during exertional or spontaneous angina by many workers (e.g. Maloy et al. 1968, Wiener et al. 1968, Parker et al. 1969 b, O'Brien et al. 1969) During exercise the cardiac response involves, in addition to simple tachycardia, sympathetic stimulation and the operation of the Frank-Starling mechanism.

Tachycardia during exercise is one of the three adaptative mechanisms. During maximal muscular exercise all three are required while during submaximal exercise cardiac output rises even when one or two of these influences are blocked (Braunwald et al. 1967). *The effects of tachycardia* and the increased sympathetic ctivity contribute to the decrease in end-diastolic dimensions

opposing the Frank-Starling effect on ventricular end-diastolic fiber length. During violent exertion the ventricular end-diastolic volume will increase, accompanying an increase in stroke volume. However this rise in stroke volume has not been seen by all investigators. At the onset of exercise there is a transitional increase in stroke volume both in the supine and sitting position. With increasing work loads in the sitting position stroke volume tends to level off except in old age groups (Ekelund and Holmgren 1967 Julius et al. 1967). In the supine position stroke volume is larger at every level of exercise, but the changes are minimal and unpredictable. The smaller stroke volume in the erect position results most probably from a peripheral pooling of blood due to the influence of gravity The decrease in venous return, filling pressure and volume causes a fall in the preload of the left ventricle. The mean arterial pressure will rise when standing, less when sitting, after the change from supine to erect position (Ward et al. 1966). The increase in total peripheral resistance, and in the afterload of the left ventricle, is usually not enough to prevent a net reduction in left ventricular stroke work.

In angina pectoris patients the exertion in the supine position over a critical level will consistently induce an elevation of the left ventricular end-diastolic pressure, which is far larger than that induced by atrial pacing. Stroke volume and stroke work will increase, in contrast to the decline usually observed with paced tachycardia. Maloy et al (1968) studied hemodynamic changes at the moment of the onset of anginal pain. He compared both techniques, and found the tension time index to be non-significantly different with both methods. However angina was induced at heart rate 104/min. on the average with exercise and at 124/min. with atrial pacing. In a similar study O'Brien et al. (1969) also found heart rates to be lower in each patient for angina during exercise The tension-time index and the left ventricular peak dp/dt were, however significantly higher The left ventricular end-diastolic pressure was abnormally elevated in each patient during supine exercise, whereas it was normal at the onset of angina provoked by pacing The total diastolic time per minute was greater during pacing Therefore, the longer diastolic filling period cannot explain the higher TTI during exercise. Two patients out of nine did not develop angina by pacing although it was readily provoked by exercise.

Hemodynamic studies of angina in the erect position are very rare There are probably no such studies made with the atrial pacing technique. Epstein et al. (1967) studied hemodynamic parameters during moderate and heavy upright exercise in six normal untrained men and in 21 patients with various types of cardiac disease. No patients with angina pectoris were studied, however In the patients stroke volume was found not to change or actually to fall as the intensity of the exercise increased. The mean systemic arterial pressure fell in four of the 19 patients. Total peripheral resistance decreased in the patients as in the six normal persons. The patients showed markedly greater arteriovenous O_2-difference and the authors found the cardiac index at a pulmonary arterial O_2-saturation of 30 per cent to be a reliable and sensitive indicator distinguishing an abnormal response from the normal. Malmcrona and Varnauskas (1964) studied hemodynamics in patients at the end of their convalescence from myocardial infarction during an exercise of 200 kpm/min. in the seated position. They found that patients with abnormally low resting cardiac outputs performed surprisingly well during work. The stroke volume response to work was normal even if stroke volume at a given work load was lower than in controls (Malmcrona 1964) Similar studies which had been continued until the onset of chest pain are not known to the present author

ECG-SIGNS OF MYOCARDIAL HYPOXIA

Accompanying myocardial hypoxia typical changes appear in the electrocardiogram, mainly in the part representing ventricular

repolarization i.e. the ST segment and the T wave. These changes are, however very unspecific and it can never be stated quite positively that any particular change in repolarization is a sign of myocardial hypoxia. However if the ECG is normal at rest, and the patient during exercise develops a chest pain of the angina pectoris type, and the ECG at approximately the same time shows a certain kind of depression of the ST segment that disappears a few minutes after the exercise has been ended, then the result of the exercise test is quite definitely positive. It is very often the case during the test that the chest pain is very slight and atypical in character and the ECG-changes too are minimal and not typical. In these cases interpreting the result is difficult and the standpoint uncertain. In unclear cases the human factor becomes determinative as well and the inter-observer variation great.

Ever since 1928, when Feil and Siegel for the first time delineated the change in the ST segment due to exercise, and Goldhammer and Scherf suggested an electrocardiographic exercise test (1932), attempts have been made to develop the method and especially to improve specificity and sensitivity The reviews by Bruce and Hornsten (1969) and Simonson (1970) give a good idea of this work and the problems, which are still to a great extent unsolved. The concluding comment of Bruce and Hornsten is that in the first 50 years of stress electrocardiography of ischemic heart disease, only the more superficial aspects have been delineated In spite of all the developmental work the central problems of electrocardiography are still inadequately worked out. A more comprehensive discussion of electrocardiographical questions goes beyond the scope and subject of this paper It is possible, however to offer a few observations and comments.

The most essential recent improvements in the exercise-ECG technique have, as Sheffield et al. (1969) state, been the increase in the exercise stress involved, the introduction of ECG monitoring during exercise and the application of computer technology to the evaluation of the ECG signal. To these could be added the quantitative approach to the exercise test, in which the amount of work done and the load at each moment are known, and the polyparametric registration procedure in which at least the changes in heart rate and arterial blood pressure are observed simultaneously and possibly the changes in pulmonary pressure as well (by the use of a floating microcatheter). Electrocardiography has indirectly profited from the advancement of other methods of study ECG-changes can nowadays be compared with the recordings of the anatomical condition of the coronary vessels made by means of coronary angiography and of the functional condition with flow measurements. Also a direct approach in connection with operations on the coronary arteries gives useful feed-back information.

The quantitative analysis of the ECG itself obviously provides information about the reliability of the findings and possibly also about the approximate degree of narrowing in the coronary vessels. By using the usual twelve leads an attempt has been made to classify the ECG-findings in accordance with the extent of ST-depression, for instance in the Minnesota code (Blackburn et al. 1960 Rose and Blackburn 1968) and in the Scandinavian code developed from the former by the Scandinavian Committee on ECG Classification (1967).

Both the extent and type of ST changes depend on the intensity and duration of the test, as Kaijser (1966) and Cherchi (1969) have shown. A third important determinant for the ST depression is the exercise tachycardia. As Simonson (1970) says, the heart rate should be considered in the criteria for ST depression for several reasons: (1) shortening of the plateau of the action currents of the individual fibers will produce a ST

depression, (2) tachycardia increases the P wave and T_a, producing junctional ST depression, and (3) most important is the in crease of myocardial O_2 consumption with an increase in heart rate.

A strict standardization of all these factors (intensity duration and heart rate) was also the main reason for the developmental work on the pulse-conducted exercise-ECG test by the present author since 1967 In the PCT X test every person will attain the same heart rate increase after the same working time on an intensity level which is automatically matched in regard to the individual s capability

Automatic computer analysis of the ECG has, particularly where exercise-electrocardiography is concerned, proved to be an indispensable method (Blomqvist 1965 Smith and Wherry 1966 Sheffield et al. 1969). This usually means changing over to orthogonal X, Y Z leads and spatial analysis of the changes as vector quantities with a direction and magnitude, the former indicated as azimuth and elevation, whereat the point of the vector is sited on the surface of a sphere. Any final solution regarding the method of analyzing the changes of the ST segment has probably not been obtained. Any mean instantaneous vector from X, Y and Z leads during the ST period, or the time integral of the whole ST segment, may be considered. The first derivate of the voltage, dV dT will very likely prove to be better than voltage itself.

Even after the best way of doing the ECG analysis has been found, there still remain large scale population studies to be done. The limits of a normal exercise response in healthy people must be determinated, taking their body build, fat deposits heart position, possible lung emphysema etc. into consideration. In principle the varying degree of narrowing in the three coronary arteries, separately and together use different findings. A single r analysis would, however with variations, be acceptable everywhere. The methods of automatic analysis eliminate muscle and other random noise from the recordings (Rautaharju and Blackburn 1965) and also eliminate the beat to beat variation either with average or median computation (Sheffield et al. 1969) Also the considerable interobserver variation in interpreting the exercise — ECG by conventional methods (Blackburn et al. 1968) the least precise aspect of the exercise test as Sheffield and associates say can thereby be eliminated If an on line analysis is being used the supervision required for the sake of safety can, to a great extent, be carried out by means of a computer A short review with personal comments on the value and cost of computerized ECG-interpretation is also offered by Friedberg (1970)

OTHER METHODS IN EXAMINATION OF CORONARY CIRCULATION

Instead of exercise or atrial pacing other means can be used to provoke ischemia in an ECG test. Examples are the isoproterenol and hypoxia stress test described below Coronary venous blood samples can be analyzed and an ischemic state demonstrated directly without an ECG registration. The visualization of coronary vessels is nowadays possible with the angiographic roentgen technique as also an indirect determination of coronary or myocardial blood flow with radioactive substances. All these methods which yield parallel information with the exercise-ECG are briefly reviewed below

Instead f exercise other means can be used to provoke an ischemia. An infusion of *sympathomimetic agents* mostly isoproterenol, has been used in patients with poor exercise tolerance and also in multiparametric laboratory studies where the movements of the patient might disturb the registration procedure, e.g. by Krasnow and 63), Cohen et al. (1965) and Herman) in angina pectoris patients, and by L (1964) and Ross et al. (1965) in bjects.

Hypoxia stress tests, with inhalation of a gas mixture of, e.g. *10 per cent oxygen and 90 per* cent nitrogen, has, since Levy et al. (1941), been used on a limited scale, more recently by Kassebaum and Griswold (Kassebaum et al. 1968, 1969). Critical comments on this technique have been presented by Lepeschkin (1960), Bruce and Hornsten (1969) and Roskamm (1971) and more profound review has been made by Simonson (1970). Hypoxia testing has a drawback common with catecholamine or exercise testing. The effects on the central circulation are complex and the myocardial oxygen consumption is often affected in an unpredictable manner. This drawback is avoided only when the heart is paced electrically as described earlier.

In clinical catheterization studies the method of *sampling coronary sinus blood* has been used to indicate the ischemia as shift to anaerobic metabolism resulting in a decrease in lactate extraction or in production of »excess» lactate (*Krasnow and Gorlin 1963, Parker et al. 1969 a, b*). Harman et al. (1967) have studied the regional lactate pattern by multiple site coronary venous sampling. Other changes in coronary sinus blood are decrease in blood pH due to a hydrogen ion efflux accompanying a potassium loss and the lactic acid production. Also the pO_2 of the coronary sinus blood is low during ischemia. *After the ischemic state there is prompt fall* in the elevated coronary sinus potassium level for several minutes until it is below the arterial level which indicates potassium uptake (Case et al. 1969). The most suitable method for provoking ischemia in this kind of study is naturally atrial or coronary sinus pacing.

The measurement of coronary or myocardial flow is possible both at rest and during exercise using variety of methods, which are all based on the indicator dilution principle. The theoretical limitations of these methods, however are great, and the procedures are technically complicated. Benchimol et al. (1968) injected indocyanine green into the ostia of both coronary arteries selectively and obtained dye-dilution curves with a catheter in the pulmonary artery. A more common technique, adopted in order to avoid the mixing of the dye with the total venous return, is the use of coronary sinus sampling. Only the left coronary flow can then be measured, however because the right coronary is only partly drained by the coronary sinus. In addition about one-fifth of the left coronary flow is missed with this technique for the same reason, and about one-fifth of the rest is mixed with the right coronary flow (Bachmann and Zoich with remarks to the paper by Buttabauser 1971).

The myocardial flow can be measured with an indicator which leaves the vascular space and enters the myocardium. Gas indicators, nitrous oxide, argon, ^{85}krypton or ^{133}xenon, are often used, because about 95 per cent of the recirculation effect is eliminated by exhalation through the lungs. Precordial scintillation detection can be *used separately for the estimation* of right and left myocardial flow by injecting a radioactive substance into the respective coronary arteries. According to Friesinger (1971) this technique does not distinguish the healthy person from the diseased one in the resting state. Holmberg (1971) has used the ^{133}xenon method in exercise with a non-selective coronary injection. He states that this method has a limited value in patients with coronary disease, because it gives only the value of the dominating flow. Rowe et al. (1969) found that the nitrous oxide saturation technique with coronary sinus sampling *could not separate subjects* with normal coronary arteries from those with coronary artery disease, nor did it correlate with the severity of the coronary artery disease demonstrated by angiography. Although their study was done with resting patients. Rowe and associates refer in the discussion to four other studies, where by exercising or by giving isoproterenol or epinephrine *the coronary flow increased as much* or more in subjects with presumed coronary artery disease as in controls. The absolute values of the flow measured with these methods are often not meaningful. Serial analyses of the same patients, regarding the effects of drug or surgical therapy however are valuable. These methods are practicable when used allied to coronary angiography. *The control of the heart rate by atrial pacing is* necessary in repeated examinations.

Coronary angiography has now (in 1971) established its position as both the final and the best method of studying the coronary arteries in selected patients. According to Sheldon (1971) *this method provides highly accurate means* of determining not only the presence of coronary atherosclerosis but also its significance. Finally coronary angiography provides the basis for the selection of patients for medical or surgical treatment. To this could be added that coronary angiography is also the basis for the evaluation of all other diagnostic techniques concerning coronary circulation. This applies especially to exercise-electrocardiography. The significance of ECG-changes could formerly be estimated only

by observing over a period of years patients in whom a certain change had been discovered and by finally noting if these patients developed infarcts or show a disproportionate mortality. Now it is possible to compare a certain observation in the exercise-ECG to the anatomic condition in a coronary angiogram the very next day. The instructive feed-back is considerable and at the same time it is seen how the exercise-ECG test should be done and the results interpreted in order to maximize reliability. While the correlation between the exercise-ECG and the coronary angiography has previously been rather poor this correlation has continuously improved as both methods have improved (Siitanen 1970, Kaltenbach et al. 1971). In addition, exercise-ECG is becoming more than mere electrocardiography when in the same test attention is paid quantitatively to total body and cardiac work, and possibly the increase in diastolic pulmonary pressure, caused by the exercise can be also measured, as Roskamm (1971) suggests. On the other hand, coronary angiography in its newest form is more than just a roentgenological visualization of the coronary arteries. The procedure needed just for this does not require much extra work if in the examination are also included left ventricular angiography measurement of the end-diastolic pressure and of the myocardial flow or of the metabolism both at rest and during tachycardia induced with atrial pacing.

The fact that the methods in the catherization laboratories are developing does not lessen the need for improving the mass screening tests, such as the exercise-ECG. On the contrary as the required following examinations keep getting more complicated and expensive, it becomes important also that the diagnostic reliability of the screening methods improves in such a way that the examination of suspect or uncertain cases will not unreasonably tie up the capacity of the catherization laboratories. When a widespread disease is concerned such as the coronary heart disease, in which limited resources make it difficult to help everybody the reliability of the grounds and criteria for selection is important even at the first stage of examination.

III TYPES OF EXERCISE TESTS REVIEWED

TEST PROCEDURE IN GENERAL

Four forms of physical exercise have mainly been used in the exercise-ECG tests. These are stepping up and down stairs, walking or running on a treadmill and hand cranking or pedalling an ergometer. All of these forms have their advantages. Stepping, walking, and running are familiar forms of exercise to the subjects. In the author's opinion, however, the pedal ergometer has decisive *advantages*

- the subject's trunk is almost motionless and thus it is easier to get high-quality ECG recordings, to measure blood pressure at regular intervals during the exercise and to observe and interview the subject all the time — these possibilities also enchance the safety of the test
- when the braking force and the number of revolutions are known, the mechanical power can be calculated accurately and thus the amount of external work can be compared with the cardiac work as reflected in heart rate and blood pressure, with subjective symptoms like chest pain or dyspnea and with ECG-changes this is the basis of *a quantitative exercise-ECG test*
- by regulating the braking force it is possible to change the work load instantly so that it corresponds to the subjects capacity at *each moment, as in the heart* rate regulated PCT X test.

The efficiency of effort performed on the bicycle ergometer varies at different work loads. Shephard et al. (1968) found a variation in efficiency between 19.3 % and 26.1 %. The variation decreased when the load was increased. According to Nowacki (1968) the efficiency of the ergometer pedalling was 24.15 % at 100 watt steady state and 23.78 % at maximal work load in 36 untrained *healthy men aged between twenty and forty*. Trained men were able to attain a (relative) 9 per cent higher efficiency on an average. The highest efficiency among the athletes, 27.7 %, was shown by a still active 45-year old cyclist. Some increase in efficiency with age, up to middle-age, was observed. A certain increase due to learning how to do the pedalling will also occur. Shephard (1968) *found a change of about 10 % in* the cost of unit work when the subjects pedalled a bicycle ergometer on five successive days. The rate of pedalling also affects the efficiency. 60 rev./min. has been recommended as an optimum, e.g. by Messin et al. (1968). Shephard et al. (1968) have used this rate for submaximal tests, but increased the revolutions up to 90/min. for maximum *efforts*. Schmidt (1968) used 30, 40, 50 and 60 rev./min. in a comparative study with cardiac patients. The patients themselves found 40 or 50 rev./min. most convenient. Oxygen consumption was also lowest at these rates. For these reasons it is likely that no strict standardization is possible in this respect. Young and healthy men will obviously reach their highest efficiency when pedalling at *the rate of 60* rev./min. or more. Women, older men and especially patients will prefer the rate of 40—50 rev./min. Leg fatigue is more likely when low rates are used and pains in the thigh muscles will possibly interrupt the test before general symptoms appear

The bicycle ergometer has some disadvantages. Cooper (1968) states that on a bicycle ergometer motivation determines whether a subject will push himself to reach maximum effort. A treadmill test is less dependent upon motivation. If the subject cannot or will not keep up the speed, he comes to an abrupt, sometimes embarrassed halt. According to Shephard et al. (1968) the directly measured maximum oxygen intake is 6.6 % greater during uphill treadmill running than during maximum work on the bicycle ergometer. Subjects become more easily accustomed to the treadmill than to the bicycle ergometer. Shephard and co-workers, however, prefer a set of two nine inch steps to both the treadmill and the bicycle ergometer for field tests. Each of these three modes of imposing the work load has its adherents. Generally speaking the use of the treadmill is preferred, for example, in the United States. In Europe bicycle and cranking ergometers are more common. Naturally most laboratories like to use one standardized procedure for most patients. The choice must depend on the circumstances, for instance, for the measuring of the maximum performance of young healthy people the treadmill is perhaps the most suitable means. On the other hand, the old or sick, who would experience anxiety on a treadmill or instability in step-climbing prefer pedalling an ergometer in a sitting position, particularly if the saddle is replaced by a modified armchair. For a CHD patient with a mild or moderately severe disease the ordinary bicycle ergometer with a pedalling rate of 50 rev./min. is perhaps the most appropriate method.

The body position when pedalling can be either erect or supine. The latter however is a most unnatural position for near maximal or maximal performance. Particularly in mass check ups a position resembling sitting on a bicycle is to be preferred, even though orthostatic ECG-changes and vasovagal collapses will be more common than in the supine position. The position as such is an unimportant detail. It is, however noteworthy that the ergometric results, the normal values, are dependent on the working position. A CHD patient will, in the supine position, get chest pains, and particularly dyspnea, earlier than when pedalling upright. This is because the venous return and stroke volume are larger and the wall tension and myocardial oxygen consumption greater in the recumbent position.

The intensity and duration of the test, and especially the procedure of loading are important factors and according to them exercise tests are classified into several types. Ten patterns of loading are represented in Fig. 1 with a division into *rectangular* and *triangular* types of tests with various subgroups and intermediate groups.

The concepts, rectangular and triangular are used in this text unanimously e.g. with Cherchi in Italy, Bonjer in Netherlands, Denolin, Messin and Degre in Belgium (Denolin et al. 1968, Messin et al. 1968). A different terminology has recently been suggested by the WHO working committee Andersen et al. in 1971. Rectangular loading is now »constant level load» and triangular is »continuously or almost continuously increased load». The author prefers the term »triangular» because of its brevity.

The most common or most important standardized exercise-ECG tests are reviewed and critically discussed below also in order to describe and illustrate the unique characteristics of the pulse-conducted type of loading. The main concern is the suitability of the exercise test for inducing myocardial hypoxia and for the ECG recording of this phenomenon. Some ergometric tests, principally used for measuring physical fitness, are therefore omitted.

SINGLE-LEVEL RECTANGULAR TESTS

In the single-level rectangular test the subject works under a constant load through-

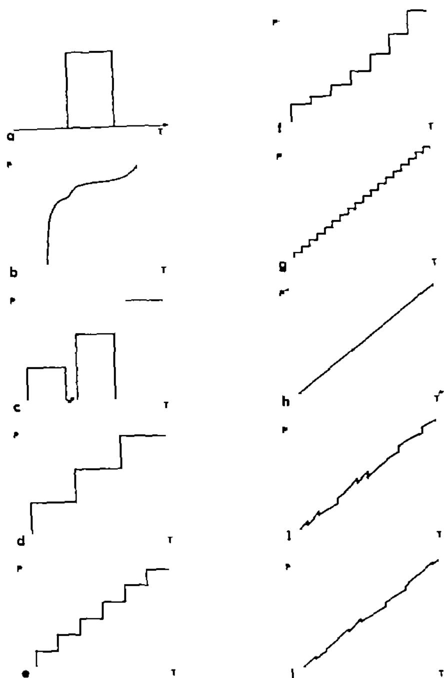


Fig. 1 Different types of ergometric exercise tests grouped according to the increase in work load or power (P) during the working time (T). The tests in figures a, c, d are of the rectangular type, in figure and f of the intermediary type and in figure g—j of the triangular type.

out the test. As a rule the working period lasts for 3—6 minutes, or until a *steady state* of adaptation is achieved and, for example, the heart rate, blood pressure, blood lactic acid concentration, tissue oxygen consumption etc. no longer change appreciably A perfectly steady state is reached only with healthy subjects, by using low exercise levels,

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SINGLE-LEVEL RECTANGULAR TESTS

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cases of angina pectoris, and on the other hand that it is too hard for severely ill coronary patients. Friedberg et al. (1962) found, by using as ST depression criterion 1 mm. or more, that the test gave 8 % false positives and 43 % false negatives with 100 subjects.

It is obvious that Master's test is a practical qualitative ECG-exercise test, in which the positive result is significant, but in which the negative result does not exclude the possibility of coronary disease. It is also obvious that the test is better than the original version, if equalization according to sex and age, but not according to body weight is used.

Other single-level tests. Blackburn et al. (1966) and Keys et al. (1970) have used in epidemiological studies in seven countries during a ten year follow-up a very similar but on theoretical and practical grounds better procedure than the Master schedule. In this test all men aged 40—59 years performed a single step test, mounting and descending for 3 minutes, 20 ascents per minute, a step 12" or 30 cm high. No ergometric results were calculated. An ECG recording was done in the supine position immediately after the test. As a part of the study U.S. railroad employees walked for 3 minutes on a treadmill at 3 m.p.h. and 5 per cent (a one in twenty) grade. Blackburn et al. (1970) compared these two tests with the original double Master two-step test and with the same two-step test performed at a fixed rate of 40 crossings by all subjects, irrespective of age, weight or sex. A 3-minute bicycle ergometer test, seated, at a pedal rate of 50 rpm against resistance giving a load of 600 kpm/min was also performed by ten male test subjects. Significant differences were found in the energy cost and heart rate response between these five testing procedures. The double Master test showed highest inter individual variability in oxygen consumption and heart rate which also on the average were highest. The variability in the treadmill walking was lowest for energy cost, that for heart rate was about the same in all constant rate procedures. The heart rate for the bicycle test was disproportionately high perhaps related to the subjects' inexperience in bicycle riding

The Harvard step test (30 ascents per minute for 5 minutes of a step 20 or 50 8 cm high) is a maximal test for most men and can be used only when studying young males. The correlation of the results, calculated from the heart rate after the test, has been found to be 0.31—0.45 with directly measured maximal oxygen uptake (Blohmke 1969).

Individual regulation of the height of the step has also been used. Hettinger and Rodahl (1960) and Gottheiner (1968) used the length of legs as the basis while van Lingen et al. (1965) used the subjects' weight. The six minute step test by Kaltenbach (1968, Kaltenbach et al. 1971) combines pulling with arm muscles with leg exercise. The range of work load possible is noticeable 300 watts and the test gives a proper loading for most clinical purposes.

Of the single level rectangular tests using bicycle ergometry the best known test is that developed by Irma and P.-O. Åstrand, in which the subject usually pedals for 6 min. such a load as is assumed to increase the heart rate to between 125—170 beats/min. The ergometric result of a submaximal test is estimated, predicted as maximal oxygen consumption by using as empirically fixed nomogram (Åstrand and Ryhming 1954). In estimating the result the subject's weight is also considered, and thus the so-called Åstrand's index is obtained. The finished tables for obtaining this have in practise led to the load becoming fixed in work load 1 2, 3 4 or 5 times 300 kpm/min. in men and 2, 3, 4, 5 or 6 times 150 kpm/min. in women. Max. VO_2 can also be calculated indirectly with newer equations by von Döbeln et al. (1963 1967) maxVO_2 =

and by continuing the exercise long enough. Thus, in practice, the steady state has to be defined as a condition in which the change, for instance per minute, is no longer substantial. Alongside the concept of a relative steady state Mallerowicz et al. (1964) and Reindell et al. (1967) have suggested the concept of ergostase.

A single-level test is usually simple and fast to perform. This kind of test, strictly standardized, is widely used in epidemiologic field surveys when the prevalence of coronary heart disease is to be compared in different populations. Most popular tests are step tests, in which the subjects lift their body weight when stepping up and down. The height of the steps and the rate of stepping are standardized. The chief disadvantage of this kind of loading is in that the work load must be relatively light so that every person can manage the test, and therefore mild or latent forms of incipient disease (the most important individual cases) are missed because of false negative results.

The most well known step test is Master's two-step test, which because of its popularity deserves a more thorough review. The problems involved in the procedure of standardization illustrate the typical difficulties with all single-level tests.

Master's two-step test. — In 1925 and 1929 Master developed his step-test as a simple means of assessing circulatory efficiency (Master and Oppenheimer 1929).

In 1942 Master et al. suggested incorporating the ECG recording in the test. Thus one of the first exercise-ECG tests was born, in which the amount of exercise was standardized. Since then this test has reached a wider use than any other corresponding test. One of the reasons for this is the simplicity of the procedure. The patient climbs two steps which are 9 inches high, gets down on the other side and turns around to do the same thing over again. The number of step rounds in 1.5 minutes is determined on the basis of sex, age and weight using given tables. The so-called double-test means that traversing the steps is continued at the same rate up to 3 minutes.

An increasing amount of criticism has been launched against this method, which generally must be regarded as being outdated. The greatest source of error and cause of criticism are the tables themselves. The number of steps given in these does not result in physiologically equivalent loads. The lighter subjects are more heavily taxed with oxygen requirements up to 38.6 ml. per minute, while the heavier men have to stand only a minimum of 19.1 ml. per minute per kilogram of body weight (Rowell et al. 1965). The same authors, using Master's steps, also did the steps at a standard rate of 40 steps in 3 minutes and the dispersal of the values was much smaller. The coefficient of variation was 3.1, while it was 8.9 when using Master's tables. A fat person also has a larger muscular mass and heart and in addition a more efficient blood circulation than a thinner person, and thus a fat person does not need equalization in the form of fewer steps as the tables indicate. The concept of equivalent physiologic stress has been presented in detail by Simonson (1961). Van Lingen et al. (1965) proved that by regulating the height of the step in linear relation to the subject's weight, the correlation between the heart rate and the work load increased at the same time as the standard error of the prediction around the regression line decreased. This correlation between the work load and the heart rate must especially be demanded from the exercise-ECG test, because the oxygen consumption of the myocardium depends, for its part, mainly on the heart rate (Sheffield et al. 1965).

The test constitutes a moderately severe exercise demanding 45 per cent of the mean maximal oxygen capacity in young men (Ford and Hellerstein 1957). This means that the test does not detect slight, developing

relative steady state, ergostase, when his pulse in an interval of 4—6 min. is increased by less than 8 beats. The authors have included in this method the actual oxygen consumption as well as the measuring of the pulse and they give the ergometric result as a quotient of these, called O_2-pulse. They have counteracted the effect on the results of the difference in size between the subjects by using the heart's roentgenological volume as an equalizing basis of comparison. Oxygen pulse is an interesting single value giving information on cardiac reserve and on the functional economy of the cardiovascular system, as has also been stated by Kirchhoff and Lauschner (1966) who have used a similar testing procedure for an early diagnosis of cardiovascular disease among German air crews.

A drawback common to both these methods is the rather long duration of the test, a minimum of 18 min., often 24 min. Furthermore, the heart rate rises very abruptly at each stage of stepping up the exercise, which is also seen in Fig. 20 The effect of this on the pedalling subject is the same as, for example, the effects on a long-distance runner when he tries to leave his competitors behind by suddenly pulling away from them. In other words, the balance, which has already been attained by the help of adaptation, is upset for a few minutes. A spell of fatigue, chest pain, agony also a rhythm disorder may in a cardiac patient be the result of an sudden unpleasant change. True it must be noted that the relative increase gets smaller for each time since this increase is always of the same size. The main advantage of this test, the attaining of a relative steady state, is perhaps immaterial from the point of view of exercise-electrocardiography It becomes useful only in complicated physiological measurings, in which there is not time to get all the recordings at once and a stabilized state is therefore an advantage. However nowadays in these examinations the technique of atrial pacing, for instance, offers even more stabilized conditions for studying particularly coronary or left heart insufficiency than prolonged exercise with a standard load. Moreover the attaining of a real steady state requires periods even longer than 6 minutes, and at high exercise levels or when sick persons are involved it is hardly attained at all.

The trend of general development, especially concerning the exercise-ECG is towards an actual shortening of the successive stages. Consequently 4 stages of 4 minutes each can be carried out in just 16 minutes. This has been recommended by among others Borg and Dahlström (1962) Siltanen (1969) suggested a similar (4 times 4 minutes) test where the load of the last stage is chosen with a special extrapolation method to give the heart rate 85 per cent of the age-dependent maximum. Research group on the methods of measurement of working capacity Toronto 1967 (see Weiner and Lourie 1969) recommended a sequence of three 4-minute loadings for the 12-min. progressive bicycle test (or four 3-minute loadings).

A kind of intermediary test between the multistage rectangular test and the triangular tests is the one where the load is increased every three minutes. Fig 1 f illustrates the maximal test used by Doan, et al. (1965, 1966), originally described by Bruce et al. (1963). In this multistage exercise capacity test the speed and grade of the treadmill are increased every 3 min. A fit person may perform 7 successive periods. The increase in average oxygen consumption exceeds one unit multiple, estimated on the basis of the values given by Kasser and Bruce (1969). Naughton and Lategola (1970) have used a test of the same kind, which was designed so that each new level induced an additional increment of energy expenditure. 2, 3 4 etc. times the basal metabolic rate until an energy demand seven times that of the resting state was attained

TRIANGULAR TESTS

Tests in which work is increased from zero by small increments or continuously can be called triangular. If the test is shortened by starting with a given basic load the test is also called trapezoidal. The most common procedure has been to increase the work load by a standard amount at one minute intervals. Thus Cherchi, in Italy, has used an increment of 10 watt/min. since 1962 (Fig. 1 g). He also noted that the results differed very slightly from each other when using an acceleration of 10 watt/1 min., 20 watt/2 min. or 30 watt/3 min. (Cherchi et al. 1966).

In Germany Müller as long ago as 1950 used an increment of 10 watt/min. in the determination of the endurance pulse index (LPI = Leistungspulsindex). The work load was increased from 5 to 100 watt in a linear way in 10 minutes. The results were calculated on the basis of the change in heart rate compared with the change in work load between different working levels. Rutenfranz (1964) presented a simplified way of calculating the LPI. The method has also been used, and critically reviewed, by Blohmke (1969).

Since according to Blohmke, 10 watt/min. is equivalent to an average oxygen consump- n f 0 2 l min. this modest increment ll ws a fairly good adaptation to exercise. Kr pping in his Vita maxima test, used an n rement of 30 watt/min. (Knipping et al. 1960). Mellerowicz used, among other methods a work load increment of 1 watt/kilogram of body weight every three minutes (Dransfeld and Mellerowicz 1962, Mellerowicz 196.).

A completely linear acceleration devised in such a way that the work load is increased non stepwise all the time has been used by Lanooy and Bonjer (1956) (Fig. 1 h). Bonjer also found that nearly identical results were obtained if the work load was increased continuously corresponding to a 10 watt/min. increase or stepwise with a 30 watt increment for every 3 minute period (Bonjer 1966, 1968) (Fig. 1 e).

In the author's pulse-conducted triangular test the work load is increased (or changed) continuously. The increment of heart rate is linear, the increase of the work load is approximately so. Fig. 1 i refers to the manually operated and Fig. 1 j to the automatic version of the PCT X test (See Ch. IV).

The standardized acceleration of the heart rate *by 5 beats/min. a minute is (see Study I, Ch. V)* achieved with a mean increment of 9.5 watt/min. for an average man of 70 kg and 6.8 watt/min. for an average woman of 60 kg. Thus it can be stated that the mean acceleration for men in the PCT X test is about the same as the 10 watt/min. used by Müller, Cherchi and Bonjer and is somewhat lighter for women. The variation is large, however, since the PCT X test, in principle, is made equally laborious for every one through changing the acceleration of loading when needed.

COMMENT ON DIFFERENT TEST METHODS

The use of a triangular test method is still quite rare. Rectangular tests are favoured both in exercise-electrocardiography and in ergometry. The reasons for this are partly traditional. For the rectangular tests have, through their long period of use, produced useful normal values applicable to different populations. The risk factors and safety of these tests are generally known. In addition the replacement of a test which operates smoothly cannot be justified unless it brings decisive advantages.

The following *advantages of the triangular tests* have generally been mentioned. The test can be begun without any preliminary information about the subject by star directly with the zero load. Everyb reach the point of maximal c provided the test is continued lon

EFFECT OF ACCELERATION COMPARISON WITH A MULTISTAGE RECTANGULAR TEST (STUDY V)

In triangular exercise tests the work load is generally increased either continuously or at short intervals. In the pulse-conducted triangular test it is the heart rate which is increased. The standard increase used by the author has been 5 beats/min. per minute. With this acceleration a gradient of 50 beats/min. (e.g. 100—150) is attained in 10 minutes. The acceleration can also be faster or slower The former saves time while the latter ensures a better adaptation, in which the subject is on the verge of steady state at each moment. Using an acceleration of 4 bts/min² the gradient 100 beats/min. (e.g 80—180), which is not unusual in young people, would require as long as 25 minutes, at which stage fatigue, and particularly psychic fatigue, may appear Conducting the test, especially the manually operated version, and observing additional parameters, such as the blood pressure and the ECG require time. As far as CHD patients are concerned, the amount of time at the supervisor's disposal for observing and specifying chest pains, dyspnea and ECG changes has an effect on safety

The standard acceleration of 5 beats/min² is a kind of compromise. It was originally chosen on the basis of deductions along the lines described above, but other accelerations were also tried. Thus nine subjects did, in addition to the normal test, one test with fast acceleration (6 bts/min²) and another with slow acceleration (4 bts/min²). The analysis of the results is presented below

The same nine subjects also did the three-stage rectangular test, described by Sjöstrand (1947) and Wahlund (1948) as one part of the series of comparative studies, the PCT X test v some other well known tests. The ergometric results in terms of work load and heart rate are compared, and some observations on adaptation and on test reliability and safety are presented.

SUBJECTS AND METHODS

Each of 9 male subjects, who were students and laboratory staff with an average age of 25 7 years (22—32 years) first did the PCT X test in the usual manner After that there followed, in random order at intervals of a few days a fast test with an acceleration of 6 bts/min² a slow test with an acceleration of 4 bts/min² and a rectangular test. In addition 7 of these 9 subjects finally did the normal PCT X test once more. With these subjects, the arithmetic mean alue of the measurements done in both normal tests has been used as the result.

For the series were intentionally chosen young people for whom it was easy to attain the heart rate of 170/min., which was regarded as the common ending point to which all the triangular tests were continued. Even on the basis of the normal PCT X test it seemed to be difficult to standardize the starting point, because only a small work load caused the pulse of some subjects to rise to the range of 110—120/min. and in two subjects respiratory arrhythmia disturbed the test at the low heart rate level. Because of this an attempt was first made to produce heart rate 120 in steady state in each subtest and the acceleration of 6, 4 or 5 bts/min² was not started until this level had been attained. In other words, the tests, with the exception of the first PCT X test, were done trapezoidally The analysis of the results has been done to correspond to the gradient 120—170 bts/min.

On the basis of the results from the PCT X test three work loads, the interrelation of which equalled 2 3 4, were chosen for each subject to do successively in the rectangular test. Each work load lasted 6 minutes as constant and the total duration of this test then amounted to 18 minutes. The loads for the group wer 560, 840 and 1120 kpm/min. on average

The methods used in all tests were the same as those described in connection with Study I. The tests were done at the same time in the afternoon or evening. The subjects were asked to prepare themselves in the same way for each test. In practice, however it turned out that complete uniformity was not reached in this respect, as the five tests came in rapid succession and, among other things, the strenuousness of the working day varied.

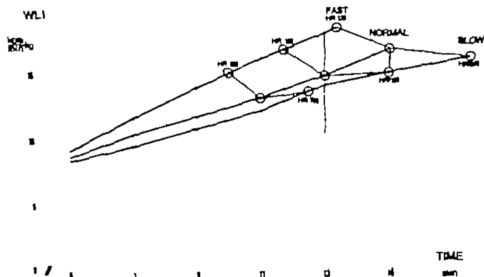


Fig. 19 Mean values of work load indexes (WLI) during three PCT X tests with fast, normal and slow heart rate accleration (6, 5 and 4 beats/min² respectively) in the same 9 male subjects.

Table 20. *Mean values and their standard errors of work load index (WLI) and total work index (TWI) during three PCT X test with different accelerations of heart rate in 9 male subjects. (wT = working time).*

Acceleration of $HR_{exp.}$		WLI at HR 150	WLI at HR 170	TWI during wT 8 min. (5th — 13th min.)			TWI from HR 120 to HR 170		
	A_1	$\bar{x} \pm SE_{\bar{x}}$	$\bar{x} \pm SE_{\bar{x}}$	$\bar{x} \pm SE_{\bar{x}}$	$\bar{x}$[1]	$\bar{x}$[2]	$\bar{x} \pm SE_{\bar{x}}$	$\bar{x}$[1]	$\bar{x}$[3]
	bts/min²	kpm/min kg⁻¹	kpm/min kg⁻¹	kpm kg⁻¹	%	%	kpm kg⁻¹	%	%
Fast	6	14.9 ± 5.5	16.3 ± 6.2	105.5 ± 3.1	113	94	116.2 ± 3.3	93	112
Normal (= A_0)	5	13.0 ± 6.5	16.6 ± 6.9	93.6 ± 4.9	100	100	124.7 ± 6.8	100	100
Slow	4	13.2 ± 7.0	16.2 ± 8.2	86.6 ± 5.4	93	116	154.6 ± 5.9	124	99

n = 9

1) $\frac{TWI_{A_1}}{TWI_{A_0}}$ 2) $\frac{A_0}{A_1} \times \frac{TWI_{A_1}}{TWI_{A_0}}$ 3) $\frac{wT_0}{wT_1} \times \frac{TWI_{A_1}}{TWI_{A_0}}$

RESULTS AND COMMENTS

The effect of acceleration. The ergometric results from the three different PCT X tests, in which the heart rate acceleration was 6, 5 and 4 bts/min² are represented in Fig 19 and in Table 20. These show that the difference between the normal and the slow acceleration (4 bts/min²) is smaller than the difference between the normal and the fast acceleration (6 bts/min²). Accordingly the curves NORMAL and SLOW illustrating the work load index as a function of time, run close to each other in the figure. Total work from heart rate 120 to heart rate 170 is approximately 24 % greater with the slow acceleration than with the *normal* acceleration. This, however is due to the longer

working time, because if the results are calculated in relation to the working time, total work is identical with both accelera tions (last column in Table 20)

With the fast acceleration the work in relation to the working time is 12 % greater than it is with the normal acceleration. The difference is significant ($P < 0.05$) One ex planation for this unexpected observation could be that even with the normal accelera tion and a 15 minute working time, the subjects become so tired that the state of fatigue has cancelled out the better adaptation. The working efficiency has probably been the same. Altogether the described phenomenon may be analogous to what Agnevik et al. (1968) have suggested regarding energy demands during running According to them there is an anaerobic metabolism in addition to the aerobic during short runs, but the relative share of the anaerobic becomes smaller as the run becomes longer In other words, the fast acceleration in the test may have caused a larger oxygen debt than the normal acceleration. Unfortunately lactic acid determinations were not made in this series.

When total work was calculated for the same 8 minute working time (5—13 minutes) the work done in connection with the fast acceleration was 13 % greater than with the normal acceleration. If in calculating the ratio the difference in heart rates, i.e. the number of heart beats corresponding to the total work, is taken into consideration, the total work achieved with the fast acceleration remains 6 / smaller than that corresponding to the normal acceleration. In other words, the efficiency of the action of the circulatory organs would, with the normal acceleration, seem to be better

On the whole the simple test procedure could not provide a comprehensive answer to the effect of the acceleration on the test and its results. Slowing up the acceleration from 5 bts/min² to 4 bts/min² would seem to be of no use, whereas the faster acceleration of 6 bts/min² might, in addition to saving time, provide good adaptation without the test being disturbed by the direct effects of fatigue to the same extent as with the normal acceleration. Further studies with regard to the 6 bts/min² acceleration are certainly needed. Particularly in mass check ups on mainly healthy persons the saving of time is of prime importance, if it can be achieved without the loss of other advantages. In clinical work again, especially in the examination of coronary patients, a more placid test proceeding must be given pref erence over saving 1—3 minutes of time.

Comparison with a multistage rectangular test. Each one of the nine subjects also did a three-stage rectangular test. The results of this test are presented in Fig 20 The results from the PCT X test, except for two subjects, have been obtained by combining the results of the tests done before and after the rectangular test. In Fig. 20 are given the heart rate values at ½—1 minute intervals for each subject. It can be noted that a sudden increase in work load causes quite *an abrupt increase in heart rate* in some subjects. In this figure is also given the curve obtained from the mean values. When comparing the changes in heart rate to those in the PCT X test, in which the rise is ex pected to be linear the difference is considerable. In practice both healthy persons and CHD patients are likely to stop the rectangular test also abruptly corresponding to the uneven acceleration of heart rate. The results and the test safety may therefore be defective when a multistage rectangular test of this kind (with only three levels of loading) is used as a maximal ergometric test or as a quantitative exercise-ECG test in CHD patients.

The heart rate attained after 6 minutes in the rectangular test was attained about 3 minutes earlier in the PCT X test. The work load values corresponding to this heart rate were almost identical. Traditionally the

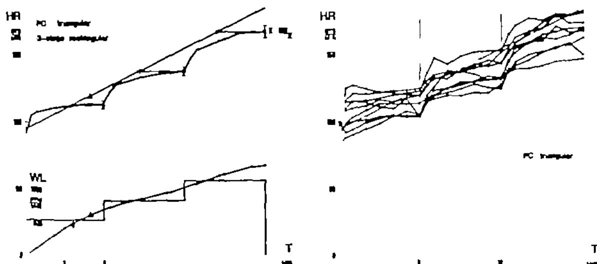


Fig. 20. On the left average heart rates and work loads during two (combined) PCT X test and three-stage rectangular test in 9 male subjects. Note that identical heart rates with the steady state heart rates in the rectangular test are reached with identical work loads in the pulse-conducted triangular test. On the right are presented individual heart rates in the rectangular test.

results of these rectangular tests are estimated according to what the heart rate is after the constant load has been maintained for 6 minutes. The PCT X test would seem to give identical results since the heart rate is the same at the same work load. If the results from this test can be stated generally then, for instance, the PWC_{170} results from the three-stage rectangular test can be compared with the results of the PCT X test without a special correction coefficient or any other correction procedure. Furthermore, the results indirectly indicate that adaptation in both tests is of the same order and that the results of the PCT X-test do not essentially differ from the results obtained in a relatively steady state.

VII PCT X TEST IN PATIENTS WITH CORONARY HEART DISEASE (STUDY VI)

The PCT X test has primarily been designed as a mass screening test for the detection of latent coronary heart disease in symptomless persons. This method can, however also be used as a part of the regular check ups on CHD patients when wanting to make an objective and quantitative examination of the patients capacity and exercise tolerance with chest pain, dyspnea, ischemic ECG-changes and arrhythmias as criteria. The test being pulse-conducted, with the modest acceleration 5 beats/min. a minute, and tri angular with the initial load being zero will prevent even a patient in poor condition from getting too strained at the early stage of the test. The test advances slowly enough to provide ample time for observing the patient's symptoms and the changes in the ECG before the decision to end the test has to be made.

Persons whose angina pectoris diagnosis is still uncertain constitute quite a large group of those studied in the exercise-ECG laboratories. The physical capacity of these persons may be quite normal and just as often substantially reduced. In these het erogeneous group the PCT X test due to its basic characteristics offers the advantage of being equally strenous in all the persons it can be completed.

In order to study how a coronary patient actually does in the PCT X test, two cohorts were chosen from the first 250 patients studied by the author by means of the PCT X method. The first group was made up of 30 men, positive cases of angina pectoris, but with no other diseases affecting physical wo king capacity To the second group were chosen 30 men with well-documented myocardial infarction who had no manifest heart failure or other disease affecting physical capacity Most of them had chest pain, exertional dyspnea or arrhythmia. The results for 18 of these patients have been published earlier in a study of the effects of alprenolol treatment (Arstila et al. 1969). 15 patients have been studied as members of a rehabilitation group In the present study however only the ergometric results of the first PCT X test, which preceded any active treatment intervention, has been reanalyzed, and special attention paid on the reasons which caused the test to be ended.

SUBJECTS

For these 60 men the requirements for their being chosen were, in detail, the following

- age 36—64 years. The average age being 51 years and the age distribution of both groups being such as shown in Table 21
- a well-documented case and t least a year f follow-up
- no medication or accidental factors were to be affective t the time of the test (the patients had been informed in advance in the same way as the subjects in Study I)
- an effort was made to exclude other diseases affecting physical capacity by means of preluminary examinations or when the test was carried out. Slightly more flexible limits for relative body weight and blood pressure were allowed here than in Study I, namely a body weight up to 40 / over the standard verage weight and a blood pressure under 190/110 in sitting position on the ergometer bicycle just before starting the pedalling
- especially those in whose case heart failure had been diagnosed and who were using

Table 21. *Age anthropometric data and ergometric results of 60 CHD patients in the PCT X test.*

		1	2	3	4	5	6	7	8	9
		Age	Body height	Body weight	Vital capacity	Peak flow rate	Heart rate at start	Heart rate at end	Working time	Last PE rating
		years	cm	kg	l	$\frac{1}{min}$	$\frac{beats}{min}$	$\frac{beats}{min}$	min	units
Angina	$\bar{x}$	51.1	173.4	78.6	3.76	473	88.4	123.4	6.9	13.6
pectoris	SD_x	7.5	7.2	11.7	0.71	82	14.7	16.2	3.8	3.8
group	CV /	14.7	4.2	14.9	19.0	17.4	16.6	13.1	55.1	27.7
	x_{min}	38	162	62	3.0	300	65	90	1.0	7
n = 30	x_{max}	64	184	106	5.2	600	115	160	16.0	19
Myocardial	$\bar{x}$	51.0	172.3	77.3	3.91	541	91.9	125.1	6.6	15.2
infarction	SD_x	7.5	6.9	9.5	0.67	78	14.7	15.1	3.2	2.9
group	CV /	14.6	3.9	12.3	17.2	14.5	15.9	12.1	48.2	18.9
	x_{min}	36	160	60	3.0	400	65	85	2.0	7
n = 30	x_{max}	63	184	95	4.8	625	116	150	13.0	19
Both	Difference $\bar{x}_{AP} - \bar{x}_{MI}$	0.04	1.0	1.3	—0.16	—68	—3.5	—1.76	0.29	—1.6
groups	*Significance* of difference	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	$\bar{x}_{AP\ MI}$	51.0	172.8	78.0	3.81	507	90.2	124.3	6.73	14.4
n = 60	$SE_{\bar{x}}$	1.0	0.9	1.4	0.16	20	1.1	2.0	0.45	0.4

digitalis or diuretics were excluded from the study Also arterial hypertension requiring treatment caused person not to be accepted to the series.
- the clinical, routine roentgenograms of all were checked. Those cases in which the heart was found greatly enlarged were excluded

Of the patients in the angina pectoris group were required both symptoms, as exertional chest pain with or without dyspnea, and ECG-changes (ST depression or arrhythmia appearing during the exercise and disappearing immediately after the exercise). A case was regarded as positive if the person had typical angina pectoris in accordance with the Rose's questionnaire (1968) and a typical ECG-change occurred in the PCT X test or if the ECG at rest showed changes in the ST T segment and typical chest pain developed in the PCT X-test. Those were excluded who showed arrhythmia even in the ECG at rest. In two patients the infarction was only subendocardial, in 28 transmural, of these anteroseptal or anterior in 24, strictly lateral in 2 and inferoposterior or strictly posterior in 22. For 9 subjects the last infarction had occurred 4—6 months back, for 7 subjects 6 months to 1 year back, for 7 subjects 1—3 years back and for 7 subjects more than 3 years back. Three had been in hospital twice due to a definite infarction and one three times.

METHODS

The methods and technical equipment were the same as those used in Study I—V The patients were more carefully observed, on the other hand the measuring, particularly of the blood pressure, had to be cut down. An effort was made to measure the blood pressure during the exercise before the subject stopped pedalling. In many cases, however arrhythmia or severe symptoms

10	11	12	13	14	15	16	17	18	19
						Blood pressure			
Last work load		Last WL/Last HR		Total work ($\sqrt{TW}$)		at start		at end	
absolute	relative	absolute	relative	absolute	relative	syst.	diast.	syst.	diast.
$\frac{kpm}{min}$	$\frac{kpm}{min}$ kg^{-1}	$\frac{kpm}{min}$	$\frac{kpm}{min}$ kg^{-1}	kpm	kpm kg^{-1}	mmHg	mmHg	mmHg	mmHg
536	6.80	4.18	0.53	46.7	5.28	146.1	91.6	186.1	88.7
303	3.89	2.19	0.26	24.8	2.76	19.6	10.9	32.6	8.41
56.6	54.2	52.3	48.2	53.2	52.2	13.5	11.9	17.5	6.10
90	1.2	0 8	0.11	11.0	1.3	120	75	148	80
1080	13.1	8 5	0.93	95.0	11.0	190	115	248	98
405	5.26	3.14	0 41	38.6	4.40	128.6	85.0	173.6	92.2
247	3.28	1.76	0.24	22.0	2.52	14.3	11.6	20.8	9.17
60.9	62.1	56.0	57.9	57 0	57.3	11.2	13.6	12.0	9.9
60	0.0	0.0	0.0	0 0	0.0	110	60	140	74
900	13.4	6.9	1.03	79.0	9 7	160	105	210	106
131	1.58	1.04	0.124	8.1	0.89	17.5	6.57	12.5	—3.58
n.s.	n.s.	P <0.05	n.s.	n.s.	n.s.	P <0.01	P <0.05	n.s.	n.s.
470	6.02	3.66	0 47	42.6	4.84	137.3	88.3	179.8	90.5
36	0.46	0.26	0.032	3.1	0.34	2.72	1.62	5.79	1 71

developed so fast that the measuring was not done.

The last PER value has generally been obtained by asking the patient after the exercise "How laborious did it feel just before the pain or the breathing difficulties started?"

As *a significant ECG-change were regarded* a 1 mm depression occuring in lead X or Y (McFee & Parungao, 1961), or in lead CM_5 1.5 mm depression, t 0.06 sec. after the J-point (i.e. at about the distance the width of QRS-complex). As significant arrhythmia was regarded the appearance of ventricular extrasystoles if only 3 normal beats occurred between the unifocal premature beats, 2 unifocal occurred successively or multifocal beats appeared. Supraventricular tachycardia or atrial fibrillation were also regarded as reason for interrupting the test.

The Student's t-test has been used as statistical method when comparing the angina pectoris group and the myocardial infarction group.

ERGOMETRIC RESULTS

In Table 21 and in Fig. 21 are presented the ergometric results of the PCT X test for the 30 angina pectoris patients and the 30 myocardial infarction patients respectively. The results have been analyzed in three ways, namely by giving the last work load at the finishing moment, the last work load divided by the last heart rate and by giving the total work. In Table 21 the results include both absolute and relative results, i.e. calculated per kilo of body weight.

The reason causing the test to be interrupted has by means of symbols also been given in Fig. 21. It should be noted that there were often two or more reasons. The test was generally not interrupted only be-

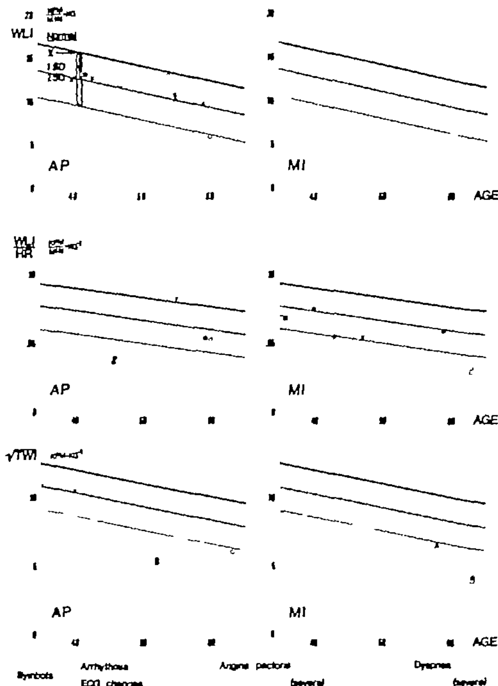


Fig. 21 Ergometric results of the PCT X test of 30 angina pectoris patients (AP) and 30 myocardial infarction patients (MI) illustrated with symbols presenting the final reason for interrupting the test. WLI = last work load per kg body weight, WLI/HR = WLI per last heart rate, √TWI = square root of total work as divided by body weight.

cause of an ECG-change, chest pain, or other symptoms were required in addition. Furthermore a slight chest pain alone was not sufficient but the test was continued until the pain was moderately severe or arrhythmia or a definite depression in the ST segment occurred. In the infarct group, however the test was interrupted a few times

for reasons of caution at the occurrence of only slight pain. In both groups special attention was paid to which of the two occurred first, the change in the ECG on the oscilloscope or the patient's complaint about chest pain or about a prodromal symptom (neck or jaw pain or left arm pain).

In the angina pectoris group both the pain and a significant ECG-change occurred in 23 cases and of these the ECG-change occurred first in eight cases, the pain first in eight and both simultaneously in seven cases. In the myocardial infarction group both occurred in just ten cases and of these the ECG-change in five, the pain in one and both simultaneously in four cases. Solely a significant change in the ECG occurred in eight, of which five of arrhythmia. Only chest pain or dyspnea occurred in twelve cases.

As can be seen in Table 21 there was no significant difference in the anthropometric dimensions between the groups. When starting the pedalling the blood pressure was significantly lower in the infarct group, in which the heart rate was 3.5 beats/min. faster on an average. It should be noted that the pressure and the pulse of the subjects were measured in a position which is closer to standing than to sitting. The infarct patients worked for 6.6 min. on an average and the angina pectoris patients for 6.9 min., yet the infarct patients found the working more laborious (in the MI group was PER 15.2, in the AP group it was 13.6). The last work load and the total work nevertheless remained smaller the only statistically significant difference appearing in the absolute values of the last work load/last heart rate ($P < 0.05$).

In Fig 21 are also presented the regression lines for the normal healthy men and the limits of 1 and 2 S.D below the mean (corresponding to the average age of 41.4 years in the normal subject material). In Table 12 numbers 1 5 and 9 are the regression formulas. No patient (Fig 21) reached the work load (WLI 19) or the total work ($\sqrt{\text{TWI}}$ 19) corresponding to the mean of the normal subject material. On the other hand WLI/HR poorly separates the healthy subjects from the CHD patients, especially in the angina pectoris group This is best accomplished by $\sqrt{\text{TWI}}$ since only 6 subjects in the AP group and 3 in the MI group attained the limit of mean $\pm$ 2 SD

COMMENTS ON ERGOMETRIC RESULTS

From the point of view of making an evaluation of the PCT X test's design and validity it was vital to study the progress of the PCT X test in a typical angina pectoris and a typical myocardial infarction group. The test clearly proved how important it is that the start is easy and that there is enough time available for the early stage. Even now the patients were able to work only for an average of 6.7 min. and attained a heart rate of 124.3 beats/min. on an average at the end of the test. The lowest values at the moment of interruption were just 90 and 85 beats/min. Three patients in the infarct group developed chest pain even from pedalling the bicycle without any load, their pulse increasing with at least 10 beats when the working time was 2 min. Altogether the ergometric results were very widely dispersed. This is only natural since the symptoms do not appear in some patients until at the utmost exertion and in others the pain may appear at rest. In addition it should be noted that a subjective report of pain was often used as the criterion for ending the test in the MI group for reasons of safety In this way the patient himself could also stop the test. The motivation for doing the test differed a great deal among different patients. A part of them came to the test because they wanted to show that they were still capable of working (a positive pre-attitude). Others planned

Tabl 12. *Complications during and after exercise in the PCT X test performed by 60 CHD patients and 236 healthy subjects. 14 subjects excluded from Study I because of ECG changes or severe symptoms during a d/ r after the test a e dealt with separately Code numbers efer to the Minnesota cod by the Sca dinavian Committee n ECG Classification (Stockholm 1967) with some completion.*

Complications in the PCT X tests during and or after exercise	Subjects	Angina pectoris patiens		Myocardial infarction patients		Healthy subjects in Study I		Healthy subjects in Study I		Subjects excluded from Study I)	
	Age range	28 — 64		36 — 63		20 — 39		40 — 59		20 — 59	
	Males, females	m.		m.		m. and f.		m. and f.		m. and f.	
	No. of tests (and subjects)	30		30		98		138		14	
		No.	/	No.	/	No.	/	No.	/	No.	/
Arrhythmias											
8.2 Ventricular tachycardia (over 100/min.)		3	10	—	—	—	—	—	—	1	4
8.3 Atrial fibrillation or flutter		—	—	—	—	—	—	—	—	—	—
8.4 Supraventricular tachycardia (over 100/min.)		—	—	1	3	—	—	—	—	1	4
8.5 Idioventricular rhythm (up to 100/min.)		1	3	—	—	—	—	—	—	—	—
8.6 A—V nodal rhythm (up to 100/min.)		—	—	—	—	—	—	1	1	—	—
Ectopic (premature) beats											
10.1 Frequent (> 5/20) multifocal VPBs		1	3	2	7	—	—	—	—	1	4
10.2 Frequent (> 5/20) unifocal VPBs		3	10	3	10	—	—	—	—	3	12
10.3 Multifocal (2—4/20) VPBs		—	—	1	3	—	—	1	1	—	—
10.4 Unifocal (2—4/20) VPBs		2	7	2	7	1	1	3	2	—	—
10.5 Occasional VPBs		7	23	5	17	6	6	16	12	—	—
10.6 Frequent (> 5/20) SvPBs		1	3	2	7	1	1	3	2	—	—
10.7 SvPBs (2—4/20)		3	10	3	10	3	3	2	1	—	—
10.8 *Occasional SvPBs*		4	13	7	23	5	5	8	6	—	—
10.9 Combination of any 1—4 and ny of 6 and 7			7	4	13	1	1	2	1	1	4

Other signs and symptoms *)

12.1.0 Severe and prolonged (> 15 min.) angina pectoris	1	3	—	—	—	—	—	—	—	—
12.1.1 Severe angina pectoris during and after exercise	4	13	2	7	—	—	—	—	—	—
12.1.2 Angina pectoris during and after exercise	6	20	5	17	—	—	—	—	—	—
12.1.3 Angina pectoris during exercise	11	37	7	23	—	—	—	—	—	—
12.2.0 Probable angina pectoris during or after exercise	2	7	5	17	—	—	2	1	3	12
12.2.1 Upper pectoral, neck, jaw pain only	1	3	3	10	3	3	3	2	—	—
12.2.2 Left shoulder or arm pain only	3	10	6	20	1	1	2	1	—	—
12.2.3 Other chest discomfort	?	?	?	?	4	4	7	5	—	—
12.3.0 Pulmonary edema	—	—	—	—	—	—	—	—	—	—
12.3.1 Aggravation of dyspnea after exercise	1	3	4	13	—	—	—	—	2	8
12.3.2 Prolonged dyspnea after exercise	?	?	?	?	—	—	3	2	1	4
12.3.3 Attack of bronchial asthma	—	—	—	—	—	—	—	—	1	4
12.3.4 Incapacitating paroxysms of coughing	—	—	—	—	—	—	—	—	1	4
12.3.5 Hysterical hyperventilation	2	7	1	3	1	1	—	—	—	—
12.4.0 Typical claudication	1	3	—	—	—	—	1	1	2	8
12.5.0 Leg exhaustion or fatigue	?	?	?	?	?	?	?	?	?	?
12.5.1 Articular pain in legs	3	10	3	10	2	2	—	—	—	—
12.5.2 Neuralgic pain in legs	—	—	—	—	—	—	—	—	1	4
12.6.0 Syncope during exercise	—	—	—	—	—	—	—	—	1	4
12.6.1 Syncope after exercise	—	—	—	—	1	1	—	—	—	—
12.6.2 Mental confusion during or after exercise	—	—	1	3	—	—	1	1	—	—
12.6.3 Near fainting during exercise	1	3	3	10	—	—	—	—	—	—
12.6.4 Near fainting after exercise	2	7	—	—	3	3	1	1	—	—
12.6.5 Dizziness or vertigo after exercise	1	3	4	13	2	2	—	—	—	—
12.6.6 Facial pallor after exercise	2	7	1	3	1	1	3	2	—	—
12 .0 Headache (severe)	3	10	—	—	5	5	4	3	1	4
12.8.0 Paralysis of any limb	—	—	—	—	—	—	—	—	—	—
12.8.1 Convulsions	—	—	1	3	—	—	—	—	—	—
12.8.2 Severe tremor	2	7	1	3	—	—	2	1	1	4
12.8.3 Ataxia	—	—	4	13	—	—	1	1	—	—

*) Up to two signs or symptoms registered if severe or noteworthy

**) Subjects were excluded if any complication interrupted the test or indicated a major disease or disorder

applying for compensation or allowances and showed that they really were incapacitated (a negative pre-attitude).

As the ergometric results were calculated in 3 different ways it turned out that especially the total work ($\sqrt{TWI}$) well distinguishes the patients from the healthy subjects. The last work load (WLI) is not so good in this respect and the quotient last work load divided by last heart rate (WLI/HR) least suitable. On the other hand it should be noted that the latter measures a fundamentally different thing than the two former ones. As the largest work load tolerated is limited by the myocardial capacity the quotient WLI/HR again reflects the patient's general physical condition, which, for instance, is affected by hospital care, inactivity orthostatic and other regulatory hemodynamic disturbances in infarct patients. Hence a statistically significant difference in the ergometric results between these two groups was noted solely with regard to this quotient. May it be pointed out that according to the author's experience the rehabilitation of the infarct patients by means of physical training improves especially the WLI/HR quotient.

COMPLICATIONS

In Table 22 are given the complications which occurred in the 60 CHD patients in the exercise tests. As far as the arrhythmias and the ectopic beats are concerned the classification is the same as in the modification of the Minnesota Code, presented by the Scandinavian Committee on ECG Classification (Stockholm 1967). The other signs and symptoms are also based on this code, but it has been slightly extended and supplemented. The complications of the 250 subjects in the PCT X test, who were considered healthy (in Study I) are given for comparison However 14 of these had to be separated into a group of their own since the severity of complications did not match the concept of a healthy normal person. These are also presented in Table 22.

Regarding both the healthy subjects and the CHD patients two ECG-leads were all the time monitored on the oscilloscope. Yet it is obvious that the supervision and, among other things, the observation of the ectopic beats were more efficient in the group of CHD patients. Code No. 8.2 ventricular tachycardia was considered to have appeared when at least 3 successive ventricular premature beats (VPB) were seen. This was also an absolute indication for stopping the test. Because of this the tachycardia of only one angina pectoris patient continued for about a minute to end immediately after the patient had been placed in a recumbent position. The patient himself felt nothing exceptional. An increase in the number of VPB during the exercise was also an indication to end the test and their number remained low On the other hand VPB appeared very often after the exercise in the CHD patients. In itself the appearance of these in CHD patients is no real complication but a phenomenon to be expected because of its high prevalence. Similarly angina pectoris was exactly what the test endeavoured to induce. In severe patients, however the pain became more severe than intended. One angina pectoris patient had chest pains more often than usually during the next week and at the routine ECG check up after a week a myocardial infarction was found. It was not necessarily caused by the test.

Often the patients seek the advice of a physician when pain attacks are increasing in number and if the ECG at rest is normal, they are often called to an exercise-ECG The consequence of this is that the test, despite the precautionary measures, sometimes is done to patients at the very moment the coronary heart disease is at

the progressive stage. When the author for example, was supervising 8 exercise tests a week in the original series of 250 patients he once received 3 cancellations one week, because the patient had been admitted to the ward for myocardial infarction 1—3 days *before* the exercise test.

Dyspnea symptoms appeared frequently in the CHD patients. Also aggravation of the *dyspnea after the exercise appeared in five* CHD patients. The symptom may primarily be caused by temporary left ventricular failure. In three patients this symptom appeared without any actual chest pain. All the patients were kept in a sitting position at rest. No one developed pulmonary edema. The dyspnea was in all the cases alleviated within 3 minutes. One patient, who had been *considered healthy developed* an apparent attack of bronchial asthma. Hyper ventilation appeared in one woman and in three CHD patients without any obvious reason. In most cases it was a sign of beginning dyspnea.

No syncope appeared during the exercise. Of the healthy subjects three turned pale and felt sick as the electrodes were being *fastened. This only happened at the early* stage of the series when they were fastened with the patient sitting on the ergometer bicycle. Later this was done with the patient sitting on an ordinary stool. One young man fainted and fell to the floor 4 minutes after the exercise. Facial pallor a decrease in blood pressure and vertigo occurred so often after the test with the patients still sitting on the ergometer that about halfway through the series a new practice was adopted. The subjects were asked to lie down as soon as the 3-minute post-exercise blood pressure and ECG had been recorded. Altogether two healthy subjects and one infarct patient were subject to this kind of spell before the critical 3 minutes.

Symptoms from the central nervous system were quite common both in the CHD patients and in the healthy subjects in the older age groups. During the exercise progressively increasing tremor and after the exercise ataxia, which for a few minutes made walking impossible, were surprisingly common. The exercise induced or aggravated headache in the healthy subjects just as often as in the CHD patients. No permanent complications from the central nervous system appeared. It should be noted that pa tients with hypertensive disease are not included in the series and the test was interrupted if the systolic blood pressure reached the value 250 mmHg All told no immediate measures of treatment requiring a physician had to be resorted to, on the other hand the good result was perhaps due to early observation of the symptoms.

Gooch and McConnell (1970) studied the incidence of transient arrhythmias during the course of submaximal treadmill testing of 713 adults. They found ectopic beats in 38 and 33 per cent and arrhythmias and/or conduction changes in 10.3 and 1.9 per cent in *398 cardiacs and 315 non-cardiacs*, respec tively All of the arrhythmias terminated spontaneously as they also did in the present study Bruce and Hornsten (1969) define the minimal incidence of major complications to be about 1 10 000 (7 myocardial infarcts and 4 deaths in 102000 exercise tests of all types involving various types of patients).

VIII COMMENTS ON PRINCIPLES OF THE PCT X TEST

Both the principles and the practice of the PCT X test are described in detail in Chapter IV. Only some complementary information and facts which emphasize the basic principles will be given here. A few drawbacks and practical difficulties entailed with the new test are given together with some means of mitigating them.

HEART RATE REGULATION

The new test will control the heart rate from the very beginning and allow only a standard increase of 5 beats/min. every minute. This is a direct approach to one of the most essential determinants of myocardial work and myocardial oxygen balance. The most important thing in the ECG-exercise test is, in the author's opinion, to control and standardize cardiac work and not the external body work, which has been the aim of many standardization committees in recent years in suggesting that predetermined work loads must be maintained for predetermined exercise periods, according to the sex, age, fitness and body weight of the subject. The loading based on given tables and the knowledge of the average reaction of average people can only by chance produce suitable conduct and control with respect to one individual. The cardiac work and heart rate may for example, be quite different for two persons even though the work load should be the same or equivalent for both on the basis of the tables.

In precise working the aim is nowadays to standardize the acceleration of the heart rate during the test. Thus Frick and Somer (1971), for instance, used atrial pacing increasing the rate by 10 beats/min. every two minutes, when assessing the effect of dipyridamole treatment in angina pectoris patients. The PCT X test uses the same acceleration with smaller steps, or operates non-stepwise. In atrial pacing a dial is simply turned for predetermined heart rate, but in the PCT X test the loading of the working organism has to be increased (or decreased) in order to attain the predetermined heart rate. This results in (1) only an approximate control of the heart rate because of the complex control mechanisms and the varying change/response, relationship, and (2) many general reactions because when regulating the heart rate with physical exercise other significant hemodynamic alterations are induced as well, e.g. an increase in venous return, in cardiac output, and in mean arterial blood pressure. Other determinants of myocardial oxygen consumption in addition to the heart rate should be controlled as well. In future it will probably be possible to extend the concept of pulse-conduction to a more complete feedback control based e.g. on the product of heart rate and systolic blood pressure.

In practice it should be noted that (1) the regulation of the heart rate with exercise is, even in the manually operated version of the PCT X test, simpler and more accurate than might be expected (See Fig. 9), and (2) the general circulatory reactions are

the same as those the patients have to face every day in connection with exertional chest pain. There remain some factors which considerably impede the carrying out of the PCT X test, if not foreseen and prevented namely an increase in heart rate caused by (3) emotional tension, (4) constant ar*rhythmias, e.g atrial fibrillation or sinus ar*rhythmia, and (5) inability to increase the heart rate during exercise, e.g complete heart block. With problems (4) and (5) it is always possible to use a conventional triangular test with a fixed acceleration of 50 kpm/min. (or 10 watts) every minute. This solution *may* also be needed if medication or some other cause in repeated tests change as they presumably do, the pulse reaction. On the other hand, this fact can be an advantage when estimating the effects of a medicine, as in the study of the effect of alprenolol on the exercise tolerance in angina pectoris patients (Arstila et al. 1969). The problem (3) of emotional tension deserves a more profound discussion.

The heart rate during rest and exercise is regulated by a complex mechanism involving both nervous and chemical factors. Cardiovascular adaptation is already initiated before starting the exercise by for example, anticipation, excitement or fear Neural pathways from motor cortex to the hypothalamus and the medulla and hence via the utonomic system to the heart pass on these responses (van Citters 1962, Morehouse and Miller 1963). As an example of anticipating reaction Agnevik and Saltin (1966) report that maximal heart rates of 200 or more beats min. were attained 20—30 seconds after the start in motocross and alpine skiing competitions. This psychic factor has its effect t the very beginning of the exercise, and other factors come into operation as the exercise continues. The first phase involves a withdrawal *of the cardioinhibitory activity mediated through* the vagus nerves. In the second phase (during muscular exercise) the beta-adrenergic activity through the sympathetic (accelerator) nerves is of greater importance. Autonomic blockage in man with tropine and beta-blockers simultaneously to gi e the intrinsic heart rate has been used during exercise, for instance by Frick et al. (1967) and by Jose et al. (1970). Beta adrenergic blockage is, however never complete in man and the effect of large amounts of catecholamines till remains. The intrinsic heart rate is also dependent on the blood temperature regardless of exercise Jose et al. suggest that increases in the IHR in exercise may represent a direct response of the myocardium to hypoxia. O'Rourke et al. (1970) have used spinal anesthesia to induce reversible sympathetic denervation in man to avoid direct myocardial effects of, for instance, propranolol. They concluded that the heart rate at rest is influenced by both sympathetic and parasympathetic activities but that the latter are predominant. The control of the heart rate during exercise is partly due to reflexes originating in the working muscles and joints. Thulesius (1969) suggests a peripheral chemoreceptor regulation in proportion to the oxygen debt and the venous lactate concentration in muscles (a theory presented by Stegeman, 1963). Clausen et al. (1970) found that even the exercise bradycardia after training is dependent on extracardiac factors in the trained muscles and could not be transferred to work performed with untrained muscle groups. Other extracardiac factors with an effect on the heart rate are the circulating adrenaline and perhaps some circulating metabolites from working muscles.

Altogether the control of the heart rate is complex and many variations of the normal mechanism are poorly understood. Three practical conclusions can, however be drawn from this. All emotional tension and excitement during the exercise test should *be avoided in order to reduce the psychic* factor as far as possible. When interpreting the ergometric results too much confidence should not be placed in the heart rate values so as to give, for example, a work load or power corresponding to some standard heart rate as a subject's physical capacity For this reason the author prefers to give the results of the PCT X test by using the PER system and by taking into account the total work. The PCT X test provides an excellent opportunity for observing deviations in the power v heart rate relationship and the curve representing this function, a byproduct in the test, (See Chapter V) may produce new and valuable information in individual cases.

PERCEIVED EXERTION RATING SYSTEM

The PER-system with perceived exertion rating originally developed by Borg (1962 a) is presented and discussed in Chapter IV. A few comments must, however be added. According to the modified scale the work load at PER 19 has been adopted as a measurement of *the maximum voluntary power* in this type of test. Total work up to PER 19 has an equivalent meaning. With most people this system works excellently but there about one person in ten does not understand the idea or misuses it. Some subjects are very well aware that they can end the test by giving high values PER 17—19. Some, especially young men or middle-aged ones, may give PER 13—15 only up to the verge of exhaustion and then all at once PER 19. With CHD patients it was found that the PER-system provided a reasonably good idea of the degree of dyspnea while the anginal pain symptoms often appeared at rather low PER-levels. Altogether this system is surprisingly good and the author would no longer want to run any exercise tests without this standardized means of communication, which follows how the patient subjectively experiences the progress of fatigue and exhaustion.

INTERPRETATION OF TEST RESULTS

The main purpose of the new test as an exercise-ECG test is to find out whether symptoms or signs of myocardial hypoxia appear when myocardial oxygen consumption is continuously increased. The answer as to the hypoxia is *yes* or *no*. The reliability of the answer depends to a large extent on the method and criteria by which the presence or absence of hypoxia is assessed.

The reliability of the ECG information depends on the quality of the ECG-technique, the quality of the ECG-electrodes used and the way they are fastened, the quality of the ECG-apparatus, the number and choice of leads and the number and choice of recording times. Two other important factors are the person who interprets the ECG and the criteria which are used as signs of hypoxia. After the test the quality of the chest pain can be asked by means of additional questions, which concern the location of the sensation, its intensity character and duration. The experience of the test supervisor affects the reliability of the final estimate. The sensitivity of both these indicators (ECG and chest pain) is relatively good, their specificity is not so good. The same kind of ECG-changes as those caused by ischemia appear for a large variety of reasons. However if the ECG at rest is normal and the subject is not under any medication affecting ventricular repolarization, the depression of the ST segment can with greater certainty be interpreted as an ischemic change. The cause may still be other than ischemia if the subject during the test either (1) hyperventilates inducing respiratory alkalosis, or (2) experiences a disproportionate rise in blood pressure, in which case the change may be an indication of acute strain of the left ventricle, or if (3) the mobilization of the catecholamines is large, resulting in, among other things, a change in the relation between the mechanic and the electric systole, or if after the exercise appear (4) an abnormally intense reactive vagotonia or (5) an orthostatic reaction in the upright position. Altogether it is only seldom possible to be certain that the ECG-changes are definitively ischemic changes. In the same way the chest pain and dyspnea can be induced by the exercise with various other mechanisms.

The best feature of the exercise-ECG test, when it is done well, is its sensitivity. If the subject does the PCT X test in such a way that both the last heart rate and

the total work make up 90 % of the maximum predicted for people of his age and even then no chest pain or abnormal changes in the ECG appear then the possibility of a functionally significant coronary heart disease is practically excluded. (Our team has studied several controversial cases among angina pectoris patients with the clinical diagnosis of CHD but with a negative PCT X test in the above-mentioned meaning with coronary angiography So far the angiogram has been nearly normal or normal in each case.) The paucity of false negative results means that an exercise-ECG test like the PCT X test is suitable as a screening test when looking for the CHD With a negative result no further examinations are necessary The positive cases again can further be divided into possibly positive and definitely positive cases. When taking into account, however the unspecificity of the exercise-ECG tests, the definitely positive group may include some false positive cases. Care must be taken not to consider a case negative when the test could not be done or the result interpreted in a reliable way Thus when using, for example, a bicycle ergometer there are always subjects who cannot or will not pedal sufficiently hard. Non-cardiac symptoms, or simply cautiousness, may necessitate an early ending. Other diseases, medication, or faulty preparation often interfere with the test result. Sometimes by repeating the test later it is possible to attain a reliable negative or positive result. Yet there remain a number of cases in which the exercise-ECG test cannot provide an answer to the question of myocardial hypoxia in these, and preferably in all the positive cases as well, examination by other methods, like atrial pacing or coronary angiography is advisable.

The total information obtained about the subjects in the PCT X tests is considerable. As an ergometric test it allows a subject's physical capacity to be measured under laboratory conditions. The total work done in the test or the maximum power attained (the last work load) indicates the capacity for short term physical work. The quotient last power divided by last heart rate is a good estimate of the physical fitness. This quantity measures the external work per one heartbeat, and the effect of regular physical training, for example, is well reflected in an improvement of this efficiency ratio.

During the PCT X test the blood pressure is usually measured at fixed intervals. Some subjects' blood pressure, normal at rest, rises disproportionately under even slight exertion. The pressure of others may under exertion *rise* less than what is average. On the basis of the test it is possible to divide people into different reaction types in this respect The practical importance of these observations has so far not been settled. The product of systolic blood pressure and heart rate reflects the oxygen consumption of the myocardium, and, especially as far as patients with CHD are concerned, the measurement of this product is useful both in itself and after treatment interventions.

Altogether the PCT X test is rather versatile. Because of the unspecifity of this kind of test, it often happens that an answer *cannot* be given *to the question as to* whether a person is suffering from coronary heart disease or not. Even in these cases all the other information is of such value that the test is well worth the effort.

IX SUMMARY

When an electrocardiogram (ECG) is recorded during or after exercise changes typically indicating coronary heart disease (CHD) are more often observed than when the ECG is recorded at rest, especially if the disease is latent or incipient. The exercise increases the oxygen consumption of the myocardium, and chest pain or ECG-changes are likely to appear if the myocardial oxygen supply is insufficient in relation to the demand. The pulse-conducted triangular exercise-ECG test (PCT X test) is a new dualpurpose test to assess (1) myocardial oxygen balance and (2) physical working power during exertion. The test differs fundamentally from all previously described exercise-ECG tests in that instead of the work load it is the heart rate which is standardized from moment to moment during the working period. This is done because the heart rate is one of the chief determinants of the myocardial oxygen consumption (MVO_2) whereas the relationship of external power (work load) to MVO_2 is only roughly parallel. When the aim of a test is to provoke myocardial hypoxia (and this is the aim of any exercise-ECG test) a controlled and non-stepwise increase of heart rate (and MVO_2) is most important for test safety and reliability

In practice the subject's (or patient's) pulse reaction is used to regulate the acceleration of the work load. The response to a load in the form of an increased heart rate affects the amount of load at the next moment through a feed-back mechanism. The author has used the acceleration of 5 beats/min. a minute as a standard. In this way and when using for example heart rate 90 beats/min. as the starting point, ECG_{110}, ECG_{130}, ECG_{150} etc. are obtained when the ECG is recorded at 4 minute intervals. The ECG recorded this way is especially suitable for automatic analysis by a computer because of identical R R intervals. For a given heart rate (e.g. HR 150 at ECG_{150}) every person has worked the same time (since HR 90) and is equally stressed subjectively as far as heart rate is an indicator of the actual stress. This enhances the interindividual comparability of the results. The new test adapts itself to all, from sportsmen to cardiac patients. No preliminary knowledge concerning the sex, age, size, fitness or health of the subject is necessary for starting the test (though information is useful for judging when to end it) and the examination of very dissimilar people is simple. Everybody has the necessary time at his disposal for adaptation. The repeatability of the results approaches therefore that of the so-called steady state tests with fixed work loads for 4—6 minutes. This means also that blood pressure BP_{110}, BP_{130}, BP_{150} etc., and other possible parameters, are reliable and valid values.

What has been said above is a logical consequence of the basic principle of the new test. The theoretical model leaves, however many practical problems to be solved. Because of this the author has analyzed the progress and the results of the PCT X test in 250 healthy subjects aged between 20 and 60, and in 30 angina pectoris and 30 myocardial infarction patients chosen from 250 examined. Some alternative ways and criteria to end the test and to calculate and express the ergometric results are ana-

lyzed and presented. In addition the PCT X test has in four experimental studies been compared with some other tests, and the validity and reliability of the new test was investigated as well. Normal ergometric values for sedentary healthy adults have been calculated on the basis of the results of 154 men and 82 women. These values provide reference figures for assessing the physical performance capacity of individuals under standard stress of brief duration.

Some other essential findings, conclusions and recommendations can be listed as follows.

The PCT X test can be done with all kinds of ergometers. The bicycle ergometer is recommended because of the possibility of adjusting the load instantly.

In a triangular test power (P) increases linearly with time (T), the work (W) according to a second-degree formula $W = f(T)^2$. The square root of work ($\sqrt{W}$) again increases linearly and square root values of total work can easily be compared with normal reference values and the differences calculated as percentages.

The end point of the test can be, and actually was, determined either by the subjective symptoms or by the objective signs of myocardial hypoxia or of some other serious disorder provoked by the exercise. If none of these appeared the subjective feeling of maximum fatigue experienced was used as a standard to indicate the voluntary maximum. This was defined with the aid of the author's modification of a scale by G. Borg, where perceived exertion ratings PER 0—20 mark the increase in fatigue, PER 19 being the voluntary maximum. Some extrapolation, assuming one degree to equal 5 per cent, was found possible in cases where the subject wanted to end the test at a submaximal level, for example at PER 17.

When the results of the PCT X test were compared with those of two other maximum tests with 30 healthy men, correlation coefficient 0.79 was found for the work load at PER 19, 0.73 for the work load at 90 % of the maximal heart rate predicted by age, 0.80 for the last work load divided by the last heart rate, and 0.72 for the total work at PER 19. When the results of the PCT X test were compared with the maximal oxygen consumption measured separately in these men after two weeks, the corresponding correlation coefficients were 0.70, 0.50, 0.63 and 0.67. Patients with coronary heart disease were best distinguished from healthy people by the total work as ergometric measurement. The last work load divided by the last heart rate was found to be a useful indicator of the physical fitness irrespective of possible angina pectoris symptoms.

The test retest correlation coefficient for heart rate at identical work loads after two week intervals was found to be 0.74. The heart rate during the retest was 4.7 beats/min. lower on average, this difference being highly significant ($P < 0.001$). The systolic blood pressure was insignificantly lower with 3.9 mmHg, the diastolic significantly so ($P < 0.02$) with 6.1 mmHg. The perceived exertion rating was 1.6 degrees lower ($P < 0.05$). (Another ergometric test between the two PCT X tests has most probably contributed to the learning effect.)

In the heart rate range 120—150 beats/min. the heart rate was at each moment about 10 beats/min. lower than a really steady state would warrant. The heart rate was, however, the same if the subject had to pedal the same load for six minutes starting directly from rest. In other words, the healthy subjects attained at the submaximal level in the PCT X test the same degree of adaptation, i.e. the same fraction of the steady state, as in a single-level rectangular test. A practical advantage is that the results can be directly compared with those obtained by Åstrand's six minute test, and the use of the Åstrand's nomogram is possible. When the ergometric results were compared with those in a three-stage rectangular test (the PWC_{170} test by Sjö-

strand and Wahlund) no significant differ ences were found. The heart rate increase was quite abrupt in the latter test as compared with the linear rise in the PCT X test.

A faster acceleration of six beats/min. a minute (instead of the standard five) was also found to provide a good adaptation. In mass screening of mostly healthy people the saving of time could possibly be achieved without disadvantages.

The manually operated technical equipment was satisfactory but even so only with a full automation of the control system and a computer analysis of the ECG will the maximal benefit from the new test be obtained. This is the aim of future developmental work on the PCT X testing procedure. With improving technical facilities more complete feed-back systems programming the increase in myocardial oxygen consumption (e g based on the product of heart rate and systolic blood pressure) are to be developed.

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APPENDIX



How light or laborious
does it feel to work just now ?

0	
1	extremely light
2	
3	very light
4	
5	LIGHT
6	
7	fairly light
8	
9	rather light
10	NEITHER LIGHT NOR LABORIOUS
11	slightly laborious
12	
13	quite laborious
14	
15	LABORIOUS
16	
17	very laborious
18	
19	extremely laborious
20	EXCEEDINGLY LABORIOUS beyond one's endurance

0	
1	[illegible] kevyt
2	
3	[illegible] kevyt
4	
5	KEVYT
6	
7	[illegible] kevyt
8	
9	[illegible] kevyt
10	[illegible] RASITTAVA
11	[illegible]
12	
13	[illegible]
14	
15	RASITTAVA
16	
17	[illegible]
18	
19	[illegible]
20	[illegible] RASITTAVA

0	
1	[illegible]
2	
3	[illegible]
4	
5	LÄTT
6	
7	[illegible]
8	
9	[illegible]
10	[illegible] LÄTT [illegible]
11	[illegible]
12	
13	[illegible]
14	
15	[illegible]
16	
17	[illegible]
18	
19	[illegible]
20	[illegible]

Fig. 1a. The scale by G. Borg for the rating of perceived exertion as modified by the author (English version)

Fig. 1b. Finnish and Swedish versions of the modified scale (photographic copy of the original tables)

Appendix table 1. Conversion of total work values (in kpm) into linea square root val es.

100	10.0	5 100	71.4	10 100	100.5	15 100	122.9
200	14.1	5 200	72.1	10 200	101.0	15 200	123.3
300	17.3	5 300	72.8	10 300	101.5	15 300	123.7
400	20.0	5 400	73.5	10 400	102.0	15 400	124.1
500	22.3	5 500	74.2	10 500	102.5	15 500	124.5
600	4.5	5 600	74.8	10 600	103.0	15 600	124.9
700	26.5	5 700	75.5	10 00	103.4	15 00	125.3
800	28.3	5 800	76.2	10 800	103.9	15 800	125.7
900	30.0	5 900	76.8	10 900	104.4	15 900	126.1
1 000	31.6	6 000	77.5	11 000	104.9	16 000	126.5
1 100	33.2	6 100	8.1	11 100	105.4	16 100	126.9
1 200	34.6	6 200	8.7	11 200	105.8	16 200	127.3
1 300	36.1	6 300	79.4	11 300	106.3	16 300	127.7
1 400	37.4	6 400	80.0	11 400	106.8	16 400	128.1
1 500	38.7	6 500	80.6	11 500	107.2	16 500	128.5
1 600	40.0	6 600	81.2	11 600	107.7	16 600	128.8
1 700	41.	6 700	81.9	11 700	108.2	16 700	129.2
1 800	42.4	6 800	82.5	11 800	108.6	16 800	129.6
1 900	43.6	6 900	83.1	11 900	109.1	16 900	130.0
2 000	44.7	7 000	83.7	12 000	109.5	17 000	130.4
2 100	45.8	7 100	84.3	12 100	110.0	17 100	130.8
2 200	46.9	7 200	84.9	12 200	110.5	17 200	131.1
2 300	48.0	7 300	85.4	12 300	110.9	17 300	131.5
2 400	49.0	7 400	86.0	12 400	111.4	17 400	131.9
2 500	50.0	7 500	86.6	12 500	111.8	17 500	132.3
600	51.0	7 600	87.2	12 600	112.2	17 600	132.7
00	52.0	7 700	87.7	12 700	112.7	17 700	133.0
2 800	52.9	7 800	88.3	12 800	113.1	17 800	133.4
900	53.9	7 900	88.9	12 900	113.6	17 900	133.8
3 000	54.8	8 000	89.4	13 000	114.0	18 000	134.1
3 100	55.7	8 100	90.0	13 100	114.5	18 100	134.5
3 200	56.6	8 200	90.6	13 200	114.9	18 200	134.9
3 300	57.4	8 300	91.1	13 300	115.3	18 300	135.3
3 400	58.3	8 400	91.7	13 400	115.8	18 400	135.6
3 500	59.2	8 500	92.2	13 500	116.2	18 500	136.0
3 600	60.0	8 600	92.7	13 600	116.6	18 600	136.4
3 00	60.8	8 700	93.3	13 00	117.0	18 00	136.7
3 800	61.6	8 800	93.8	13 800	117.4	18 800	137.1
3 900	62.5	8 900	94.3	13 900	117.9	18 900	137.5
4 000	63.2	9 000	94.9	14 000	118.3	19 000	137.8
4 100	64.0	9 100	95.4	14 100	118.7	19 100	138.2
4 200	64.8	9 200	95.9	14 00	119.2	19 200	138.6
4 300	65.6	9 300	96.4	14 300	119.6	19 300	138.9
4 400	66.3	9 400	97.0	14 400	120.0	19 400	139.3
4 500	67.1	9 500	97.5	14 500	120.4	19 500	139.6
4 600	67.8	9 600	98.0	14 600	120.8	19 600	140.0
4 00	68.6	9 00	98.5	14 00	121.2	19 700	140.4
4 800	69.3	9 800	99.0	14 800	121.7	19 800	140.7
4 900	0 0	9 900	99.5	14 900	122.1	19 900	141.1
5 000	0.7	10 000	100.0	15 000	122.5	20 000	141.4

Appendix tables 2 and 3. *N rmal mean values of work load at perceived exertion rating (PER) 19 for males and females.*

WL 19

Age	20—24	25—29	30—34	35—39	40—44	45—49	50—54	55—59	60—64
Weight					MALES				
60— 61	1 310	1 250	1 190	1 120	1 060	1 000	930	870	810
62— 63	1 330	1 260	1 200	1 140	1 080	1 010	950	890	820
64— 65	1 340	1 280	1 220	1 150	1 090	1 030	970	900	840
66— 67	1 360	1 300	1 230	1 170	1 110	1 050	980	920	860
68— 69	1 380	1 310	1 250	1 190	1 120	1 060	1 000	940	870
70— 71	1 390	1 330	1 270	1 200	1 140	1 080	1 010	950	890
72— 73	1 410	1 350	1 280	1 220	1 160	1 090	1 030	970	900
74— 75	1 430	1 360	1 300	1 240	1 170	1 110	1 050	980	920
76— 77	1 440	1 380	1 320	1 250	1 190	1 130	1 060	1 000	940
78— 79	1 460	1 390	1 330	1 270	1 210	1 140	1 080	1 020	950
80— 81	1 470	1 410	1 350	1 280	1 220	1 160	1 100	1 030	970
82— 83	1 490	1 430	1 360	1 300	1 240	1 180	1 110	1 050	990
84— 85	1 510	1 440	1 380	1 320	1 250	1 190	1 130	1 070	1 000
86— 87	1 520	1 460	1 400	1 330	1 270	1 210	1 140	1 080	1 020
88— 89	1 540	1 480	1 410	1 350	1 290	1 220	1 160	1 100	1 040
90— 91	1 560	1 490	1 430	1 370	1 300	1 240	1 180	1 110	1 050
92— 93	1 570	1 510	1 450	1 380	1 320	1 260	1 190	1 130	1 070
94— 95	1 590	1 520	1 460	1 400	1 340	1 270	1 210	1 150	1 080
96— 97	1 600	1 540	1 480	1 420	1 350	1 290	1 230	1 160	1 100
98— 99	1 620	1 560	1 490	1 430	1 370	1 310	1 240	1 180	1 120
100—101	1 640	1 570	1 510	1 450	1 380	1 320	1 260	1 200	1 130
102—103	1 650	1 590	1 530	1 460	1 400	1 340	1 270	1 210	1 150
104—105	1 670	1 610	1 540	1 480	1 420	1 350	1 290	1 220	1 170
106—107	1 690	1 620	1 560	1 500	1 430	1 370	1 310	1 240	1 180
108—109	1 700	1 640	1 580	1 510	1 450	1 390	1 320	1 260	1 200
					FEMALES				
40— 41	650	620	600	580	550	530	500	480	450
42— 43	660	640	620	590	570	540	520	500	470
44— 45	680	660	630	610	580	560	540	510	490
46— 47	700	670	650	620	600	580	550	530	500
48— 49	710	690	670	640	620	590	570	550	520
50— 51	730	710	690	660	630	610	590	560	540
52— 53	750	720	700	670	650	630	600	580	550
54— 55	760	740	720	690	670	640	620	600	570
56— 57	780	760	730	710	690	660	640	610	590
58— 59	800	770	750	720	700	680	650	630	600
60— 61	810	790	770	740	720	690	670	650	620
62— 63	830	810	780	760	730	710	690	660	640
64— 65	850	820	800	770	750	730	700	680	650
66— 67	860	840	820	790	770	740	720	700	670
68— 69	880	860	830	810	780	760	740	710	690
70— 71	900	870	850	820	800	780	750	730	700
72— 73	910	890	870	840	820	790	770	750	720
74— 75	930	910	880	860	830	810	790	760	740
76— 77	950	920	900	870	850	830	800	780	750
78— 79	960	940	920	890	870	840	820	800	770
80— 81	980	960	930	910	880	860	840	810	790
82— 83	1 000	970	950	920	900	880	850	830	800
84— 85	1 010	990	970	940	920	890	870	850	820
86— 87	1 030	1 010	980	960	930	910	890	860	840
88— 89	1 050	1 020	1 000	970	950	930	900	880	850

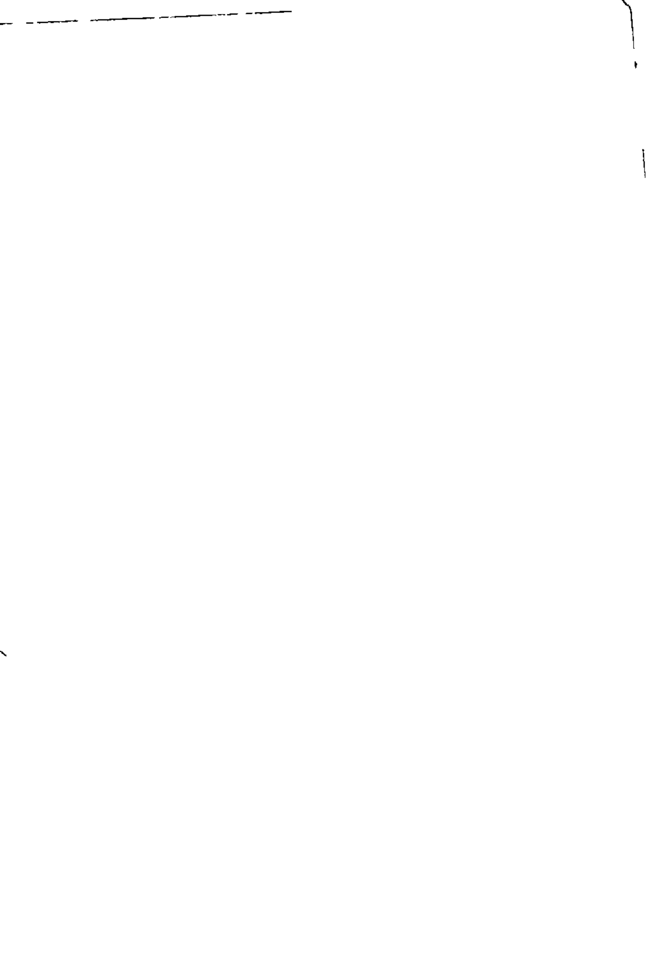
Appendix tables 4 and 5. *Normal mean values of last work load (in kpm/min) divided by last heart rate for males and females.*

Age	20—24	25—29	30—34	35—39	40—44	45—49	50—54	55—59	60—64
					WL/HR				
Weight					MALES				
60— 61	6.89	6.68	6.48	6.27	6.06	5.86	5.65	5.45	5.24
62— 63	6.98	6.78	6.57	6.36	6.16	5.95	5.75	5.54	5.33
64— 65	7.08	6.87	6.66	6.46	6.25	6.05	5.84	5.63	5.43
66— 67	7.17	6.96	6.[illegible]6	6.55	6.35	6.14	5.93	5.73	5.53
68— 69	7.[illegible]	7.06	6.85	6.65	6.44	6.23	6.03	5.82	5.62
70— 71	7.36	7.15	6.95	6.74	6.53	6.33	6.12	5.92	5.71
72— 73	7.45	7.25	7.04	6.83	6.63	6.42	6.22	6.01	5.80
74— 75	7.55	7.34	7.14	6.93	6.72	6.52	6.31	6.11	5.90
76— 77	7.64	7.44	7.23	7.02	6.82	6.61	6.41	6.[illegible]	5.99
78— 79	7.74	7.53	7.33	7.12	6.91	6.71	6.50	6.29	6.09
80— 81	7.83	7.62	7.42	7.21	7.01	6.80	6.59	6.39	6.18
82— 83	7.92	7.72	7.51	7.31	7.10	6.89	6.69	6.48	6.28
84— 85	8.02	7.81	7.61	7.40	7.19	6.99	6.78	6.58	6.37
86— 87	8.11	7.91	7.[illegible]	7.49	7.29	7.08	6.88	6.67	6.46
88— 89	8.21	8.00	7.[illegible]	7.59	7.38	7.18	6.97	6.76	6.56
90— 91	8.30	8.09	7.89	7.68	7.48	7.27	7.06	6.86	6.65
92— 93	8.39	8.19	7.98	7.[illegible]	7.57	7.36	7.16	6.95	6.75
94— 95	8.49	8.28	8.08	7.87	7.67	7.46	7.25	7.05	6.84
96— 97	8.58	8.38	8.17	7.97	7.[illegible]	7.55	7.35	7.14	6.94
98— 99	8.68	8.47	8.27	8.06	7.85	7.65	7.44	7.24	7.03
100—101	8.77	8.57	8.36	8.15	7.95	7.74	7.54	7.33	7.12
102—103	8.87	8.66	8.45	8.25	8.04	7.84	7.63	7.42	7.22
104—105	8.96	8.75	8.55	8.34	8.14	7.93	7.72	7.52	7.31
106—107	9.05	8.85	8.64	8.44	8.23	8.02	7.82	7.61	7.41
108—109	9.15	8.94	8.74	8.53	8.33	8.12	7.91	7.71	7.50
					FEMALES				
40— 41	3.46	3.40	3.34	3.29	3.23	3.17	3.11	3.06	3.00
42— 43	3.55	3.49	3.43	3.37	3.32	3.26	3.20	3.14	3.09
44— 45	3.63	3.58	3.52	3.46	3.40	3.35	3.29	3.23	3.17
46— 47	3.72	3.66	3.61	3.55	3.49	3.43	3.38	3.32	3.[illegible]
48— 49	3.81	3.75	3.69	3.64	3.58	3.52	3.46	3.41	3.35
50— 51	3.90	3.84	3.78	3.73	3.67	3.61	3.55	3.50	3.44
52— 53	3.99	3.93	3.87	3.81	3.76	3.70	3.64	3.58	3.53
54— 55	4.07	4.02	3.96	3.90	3.84	3.79	3.73	3.67	3.61
56— 57	4.16	4.10	4.05	3.99	3.93	3.87	3.82	3.76	3.70
58— 59	4.[illegible]5	4.19	4.13	4.08	4.02	3.96	3.90	3.85	3.[illegible]9
60— 61	4.34	4.[illegible]	4.22	4.17	4.11	4.05	3.99	3.94	3.88
62— 63	4.43	4.37	4.31	4.25	4.20	4.14	4.08	4.02	3.97
64— 65	4.51	4.46	4.40	4.34	4.[illegible]	4.23	4.17	4.11	4.05
66— 67	4.60	4.54	4.49	4.43	4.37	4.31	4.26	4.20	4.14
68— 69	4.69	4.63	4.57	4.52	4.46	4.40	4.34	4.29	4.23
70— 71	4.78	4.72	4.66	4.61	4.55	4.49	4.43	4.38	4.32
72— 73	4.87	4.81	4.[illegible]5	4.69	4.64	4.58	4.52	4.46	4.41
74— 75	4.95	4.90	4.84	4.78	4.72	4.67	4.61	4.55	4.49
76— 77	5.04	4.98	4.93	4.87	4.81	4.[illegible]5	4.70	4.64	4.58
78— 79	5.13	5.07	5.01	4.96	4.90	4.84	4.78	4.73	4.67
80— 81	5.22	5.16	5.10	5.05	4.99	4.93	4.87	4.82	4.[illegible]6
82— 83	5.31	5.[illegible]5	5.19	5.13	5.08	5.02	4.96	4.90	4.85
84— 85	5.39	5.34	5.28	5.22	5.16	5.11	5.05	4.99	4.93
86— 87	5.48	5.4[illegible]	5.37	5.31	5.[illegible]5	5.19	5.14	5.08	5.02
88— 89	5.57	5.51	5.45	5.40	5.34	5.[illegible]	5.22	5.17	5.11

Appendix tables 6 and 7 *Normal mean values of the square roots of total work (in kpm) at perceived exertion rating (PER) 19 for males and females.*

$\sqrt{TW\ 19}$

Age Weight	20—24	25—29	30—34	35—39	40—44	45—49	50—54	55—59	60—64
				MALES					
60— 61	115.8	111.4	107.2	103.0	99.0	94.9	90.6	86.6	82.5
62— 63	116.2	111.8	107.7	103.4	99.5	95.4	91.1	87.2	83.1
64— 65	116.6	112.7	108.6	104.4	100.0	95.9	91.7	87.7	83.7
66— 67	117.4	113.1	109.1	104.9	100.5	96.4	92.2	88.3	84.3
68— 69	117.9	114.0	109.5	105.4	101.5	97.5	93.3	88.9	84.9
70— 71	118.3	114.5	110.0	106.3	102.0	98.0	93.8	89.4	85.4
72— 73	119.2	114.9	110.9	106.8	102.5	98.5	94.3	90.0	86.0
74— 75	120.0	115.8	111.4	107.2	103.4	99.0	94.9	90.6	86.6
76— 77	120.4	116.2	112.2	107.7	103.9	99.5	95.4	91.7	87.2
78— 79	120.8	116.6	112.7	108.6	104.4	100.5	95.9	92.2	87.7
80— 81	121.7	117.4	113.1	109.1	104.9	101.0	97.0	92.7	88.3
82— 83	122.1	118.2	114.0	110.0	105.8	101.5	97.5	93.3	88.9
84— 85	122.9	118.7	114.5	110.5	106.3	102.0	98.0	93.8	90.0
86— 87	123.7	119.2	115.3	110.9	106.8	103.0	98.5	94.3	90.6
88— 89	124.1	120.0	115.8	111.8	107.7	103.4	99.0	94.9	91.1
90— 91	124.9	120.4	116.6	112.2	108.2	103.9	100.0	95.9	91.7
92— 93	125.3	121.2	117.0	113.1	108.6	104.4	100.5	96.4	92.2
94— 95	126.1	121.7	117.9	113.6	109.5	105.4	101.0	97.0	92.7
96— 97	126.5	122.5	118.3	114.0	110.0	105.8	101.6	97.5	93.3
98— 99	127.3	122.9	118.7	114.9	110.5	106.3	102.5	98.0	93.8
100—101	127.7	123.7	119.6	115.3	111.4	107.2	103.0	99.0	94.9
102—103	128.5	124.5	120.0	116.2	111.8	107.7	103.4	99.5	95.4
104—105	128.9	124.9	120.8	116.6	112.7	108.2	104.4	100.0	95.9
106—107	129.6	125.7	121.2	117.0	113.1	109.1	104.9	100.5	96.4
108—109	130.4	126.1	122.1	117.9	113.6	109.6	105.4	101.5	97.0
				FEMALES					
40— 41	75.8	73.1	70.5	67.9	65.2	62.6	60.0	57.3	54.7
42— 43	76.6	74.0	71.3	68.6	66.0	63.4	60.7	58.1	55.5
44— 45	77.3	74.7	72.0	69.4	66.8	64.1	61.5	58.8	56.2
46— 47	78.1	75.4	72.8	70.2	67.5	64.9	62.3	59.6	57.0
48— 49	78.9	76.2	73.6	70.9	68.3	65.6	63.0	60.4	57.8
50— 51	79.6	77.0	74.4	71.7	69.1	66.4	63.8	61.2	58.5
52— 53	80.4	77.7	75.1	72.5	69.9	67.2	64.6	61.9	59.2
54— 55	81.2	78.5	75.9	73.2	70.6	68.0	65.3	62.7	60.0
56— 57	81.9	79.3	76.6	74.0	71.3	68.7	66.1	63.4	60.8
58— 59	82.7	80.0	77.4	74.8	72.1	69.5	66.9	64.2	61.6
60— 61	83.4	80.8	78.2	75.5	72.9	70.3	67.6	65.0	62.4
62— 63	84.2	81.6	78.9	76.3	73.7	71.0	68.4	65.7	63.1
64— 65	85.0	82.3	79.7	77.1	74.4	71.8	69.1	66.5	63.9
66— 67	85.7	83.1	80.4	77.9	75.2	72.5	69.9	67.2	64.7
68— 69	86.5	83.8	81.3	78.6	76.0	73.3	70.6	68.0	65.4
70— 71	87.3	84.6	82.0	79.4	76.7	74.1	71.4	68.8	66.2
72— 73	88.0	85.4	82.8	80.1	77.5	74.8	72.2	69.6	66.9
74— 75	88.8	86.1	83.5	80.9	78.2	75.6	72.9	70.3	67.7
76— 77	89.6	86.9	84.3	81.7	79.0	76.3	73.7	71.1	68.4
78— 79	90.3	87.7	85.0	82.4	79.7	77.1	74.5	71.8	69.2
80— 81	91.1	88.4	85.8	83.2	80.6	77.9	75.2	72.6	70.0
82— 83	91.9	89.2	86.6	84.0	81.3	78.7	76.0	73.3	70.7
84— 85	92.6	90.0	87.4	84.7	82.0	79.4	76.8	74.2	71.5
86— 87	93.4	90.7	88.1	85.4	82.8	80.2	77.5	74.9	72.2
88— 89	94.1	91.5	88.9	86.3	83.6	80.9	78.3	75.7	73.0



ADDENDA AND ERRATA

Table 1 on page 23 presenting the latest version of the PER scale should be revised since the translation from Swedish into English does not match G. Borg's own translation

6
7 Very Very Light
8
9 Very Light
10
11 Fairly Light
12
13 Somewhat Hard
14
15 Hard
16
17 Very Hard
18
19 Very Very Hard
20

For reference see Borg, G. Edgren, B. & Marklund, G. A flexible work test with feedback system guiding the test course. Reports from the Institute of applied psychology the University of Stockholm, Nr 8, 1970.

Another paper by Borg et al. (1970) provides additional information about the exponent for perceived force (perceived pedal resistance), which was found to vary considerably from 1.4 to 2.2. For reference see Borg, G., Edstrom, C.-G. & Marklund, G. A new method to determine the exponent for perceived force in physical work. Reports from the Institute of applied psychology the University of Stockholm, N 4, 1970.

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Resumption of Work after Myocardial Infarction in Northern Finland

By Unto Vuopala

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Resumption of Work after Myocardial Infarction in Northern Finland

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Resumption of Work after Myocardial Infarction in Northern Finland

by
UNTO VUOPALA

OULU 1972

CONTENTS

RESUMPTION OF WORK AFTER MYOCARDIAL INFARCTION IN NORTHERN FINLAND

I INTRODUCTION

The methods of treating the acute phase of myocardial infarction have been improved considerably during the last few years. More and more attention has also been paid to the subsequent rehabilitation of patients recovering from myocardial infarction. It is too early however to speculate how much real benefit the patient may derive from this improved treatment. There is insufficient data to show whether the new methods help patients to live longer and improve their physical and psychic capacities, or as regards the working population, to what extent these methods can help the patients to return to active work. The medical problems alone involved here are numerous. Moreover social security schemes have been developing rapidly all over the world. While such a scheme may improve the economic wellbeing of an incapacitated person, in addition to providing other advantages, it may also change the attitudes of the convalescent and affect his motivation to resume work. The information available concerning this social aspect is not yet sufficient to be used in planning the future career of patients recovering from serious diseases such as myocardial infarction.

The extensive literature dealing with myocardial infarction contains only a few comments on the ability of patients surviving myocardial infarction to return to work. Furthermore, most of the data presented in these studies are not in accordance with the more recent notions of a modern practicing physician. The problems of patients recovering from myocardial infarction are becoming all the more urgent now that international investigations and statistics (WHO Chron 1969) have shown that increasingly younger age-groups are suffering from coronary disease and myocardial infarction. The problem is significant on the international scale, but in Finland it is particularly alarming. Investigations have shown Finland to be one of the blackest regions as regards the frequency of coronary disease (Keys 1970)

In the northern parts of the country the long distances involved make it difficult for the patient even to come to be examined, to say nothing about follow-up treatment or rehabilitation. A comparison with the south of Finland also shows the economic structure to be exceedingly simple, the level of education inadequate, and unemployment almost a constant problem. Thus the opportunities for a convalescent to return to work in this region are clearly far more resticted than those available in the southern parts of the country Experience indicates that the doctor attending the patient in hospital has a more optimistic view of the patient's future than the doctor who is responsible for the follow up treatment, and therefore more familiar with the final prognosis. There is one question which keeps recurring, viz. how much money is it advisable to invest in rehabilitating patients recovering from myocardial infarction, when there is not enough work even for the healthy people? This is one of the numerous pressing questions which remain unanswered, because no adequate surveys of the current situation exist. Such problems initiated the present project, which purposes to fill in some of the gaps in our knowledge.

II PURPOSE OF THE PRESENT WORK

The present work sets out to use material from hospital cases in the north of Finland to elucidate the current situation as regards

1) return to work after myocardial infarction and the factors affecting it, with special regard to
2) the age, sex, marital status, place of residence and occupation of the patients, as well as their working capacity at the onset of the illness.

III RESUMPTION OF WORK IN MYOCARDIAL INFARCTION CASES

The first discussion of the resumption of work after myocardial infarction was published in 1930 (Conner & Holt) Since that time there have been very few studies with this problem as their central theme. Certain difficulties are involved in the comparison of these works. First of all, enourmous progress has been made in the diagnosis of myocardial infarction since the 1930's, and the same applies to treatment and follow-up methods. It should be acknowledged, however that this medical progress has been similar and practically simultaneous everywhere. The problems of social security during the period of incapacitation on the other hand, are widely different in different countries, nor has the development, or the timing, of social security schemes been similar in all cases. What further impedes comparison is that individual raports deal with the problem of return to work from various contrasting points of view such as age, sex, occupation, severity of infarction, etc.

Table A is a summary of the most important investigations discussing return to work after myocardial infarction. The fourth column of the table is divided into three subcolumns, showing the composition of the material. Subcolumn A refers to the size of the original material where this is known. Subcolumns B and C include only the survivors. Most of the works restrict their discussion to cases for which subsequent data are available (sub-column C) The column for return to work is correspondingly divided into three parts, representing the three kinds of material and which have served as the bases for calculating the percentages returning to work.

The following survey outlines the research on return to work after myocardial infarction carried out in some other countries.

USA

The first material of Conner & Holt (1930) which originally consisted of 287 cases of myocardial infarction, only contains data for 117 survivors. Three quarters of these were under 60 years old. 93 % were in good health 3 months after the infarction, 86 % were in good health after 6 months, and 75 % after one year The term good health indicates a state of health which permits the patient to live his accustomed life and to regard himself as essentially well. Not all were entirely free from pain or other discomfort, but these symptoms were not sufficient to cause the patient to modify his mode of life or to prevent him from working A similar proportion of the patients resumed work in Cooksey s (1935) material, although the follow up periods in his work ranged from 1 to 13 years.

The material of Master & Dack (1940) which originally consisted of 415 patients with 1—4 cardiac infarctions followed up for 3 years, contained 312 people who had undergone one infarction Of these 59 % were able to resume work. Previous occupation played some role in determining whether or not the patient returned to work. Thus 84 % of the professional people, particularly physicians, and 67 % of the white collar and office

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Table A. *List of the most important works discussing return to work aft myocardial inf rction See also the text*

Authors	Year	Country	Number of patients			Age	Sex	Return to work (same or less demanding work)			Follow-up time (years)
			Total material	Survivals		mean or range	m=male f=female	Percentage of			
			A	(all) B	(know) C			A	B	C	
Conner & Holt	1930	USA	287		117	31—80	m&f			75	1
Cookley	1935	USA	53	32	3	54.2	m&f		78.1	78.1	1—13
Master & Dack	1940	USA		312		30—89	m&f		59		~3
L ne & Rosenbaum	1941	USA	372		354	53 9	m&f			75	~2.3
Rathe	1942	USA	274	219		59	m&f		16		1
Chambers	1946	USA	100		58	59	m&f			>50	1
Y t et l	1948	USA	866		361	18—38	m			55	)
Rasanen	1951	Finland	250		144	55.7	m&f			82	up to 2
Smith	1953	USA	100	30	30	33—62	m&f	27	90	90	~15.7
Cole et al.	1954	USA	390	285	244	56.7	m&f			64	2 mo.—5
Master et al	1954	USA	500		343	53.3	m&f			69.7	1—29
Ball et al.	1955	USA	342	233	233	peak 65	m&f		47	47	>1 mo.
Biörck & Trulson	1957	Sweden			85	65	m&f			80	up to 6
Pell & D'Alonzo	1958	USA	209	139	139	17—64	m&f	59		85	up to 1
Inalo et al	1958	Finla d	370		242	41—70	m&f			66.5	0.5—5
Weiss & Weiss	1958	USA			431	20—69	m			67	5
Knutsen	1960	Norway	101	86	86	<67	m			80	0.4—3 4
Malmcrona et al.	196.	Sweden	318		155	48	m&f			87	up to 8
Biörck & Wedelin	1964	Sweden	334		200	36—87	m&f			83	3.9
Sharland	1964	G-Br t.			212	<60	m			86	1
Wincott & Caird	1966	G-Brit.			63	30—70	m			88	1
Sipila	1966	Finland	1860	613	507	<65	m&f			79.2	3—18
Groden	1967	G-Brit.			61	34—64	m			84	1
Higgins & Pooler	1968	USA			83	35—55	m			80	up to 1.5
Kellermann et al.	1968	Israel	390		299	52	m&f			91	>1

) since discharg from army

workers resumed their occupations, whereas only 50—55 % of the patients doing heavier work did so Only occasionally did a patient take up a more sedentary occupation, but it was not infrequent for one to undertake a lighter job This occurred chiefly among the manual workers and labourers. More than half of the workers who resumed work took part time jobs, whereas almost all of the professional people and office workers worked full-time. Master and Dack assume that this discrepancy may be explained in part by the fact that the professional and executive classes are able to lighten their activities although they seem to be working full time and are so described. Age is naturally a factor which has a considerable effect on return to work. In Master & Dack's material taking into account all the cases regardless of whether they were first or recidive attacks, 75 % of those in their thirties and only 43 % of those in their sixties resumed work. The number of attacks suffered was similarly significant: after the first, the second and the third attack, the proportions returning to work were 59 %, 38 %, and 23 %, respectively About half of the patients who returned to work complained of pain dyspnea or weakness, symptoms which were not, however sufficient severe to cause disability

In the material of Levine & Rosenbaum (1941), altogether 75 % of the patients for whom data were available returned to work. Only 30 % were able to resume their previous occupation, 45 % assumed partial activity and 22 % moderately restricted activity Only 3 % were completely incapacitated. The authors do not specify the occupations of any of these patients. Most American authors report fairly high percentages of return to work, the figures ranging from 50 % to 90 %, but most of the papers do not contain data for all the survivors (Chambers 1946, Yater et al. 1948 b Smith 1953 Cole et al. 1954 Master et al. 1954 Ball et al. 1955 Crain & Missal 1956 Pell & D'Alonzo 1958 and 1964 Weiss & Weiss 1958 Dimond 1961 Higgins & Pooler 1968) There are, however some reports which show lower figures (Palmer 1937 Master et al. 1956).

Great Britain

Papp & Smith (1951) describe 200 consecutive cases in their study which briefly touches upon return to work, and which also includes a few cases in which the patient had suffered an infarction previously The ages of the patients ranged from 30 to 79 years. In 65 % of the cases with slight myocardial infarction normal activity was resumed. The corresponding value for the survivors of moderate myocardial infarction was 64 %. In the group with severe myocardial infarction, only 33 % of those in sedentary occupations returned to work.

The British accounts also show high percentages of patients returning to work. In Sharland's material which consisted of men under 60 years of age, 82 % had resumed work within six months (Sharland, 1964) Sharland further observes that a study of a sample of those who resumed full time work within six months indicated that while the majority tended to take it more gently than formely they were still doing a reasonable job of work and were not being »carried« by their employers. Change of job was more common among the lower social classes and those normally undertaking active or heavy work. Similar small male samples yielded results of the same order in the investigations by Wincott & Caird (1966) and Groden (1967) in Scotland.

Israel

Kellermann et al. (1968) use as their material a group of Israeli patients all under 65 years of age, with a mean age of 52 years. 90 % of the 299 patients returned to work — 89 % of the men and 88.6 % of the women 77 % of the men and 80 % of the women

resumed their previous jobs, while 11.7 % of the men and 8.6 % of the women took up another type of work. The nature of the occupation was again significant 88.5 % of those in sedentary occupations returned to work, whereas only 62.8 % of those doing physical work did so. The values for those transferring to a lighter job showed the opposite tendency 8.6 % and 23.1 %, respectively. In the category of household work, mostly composed of women, 24 out of 26 stayed in the same work. We should particularly mention the interesting assumption of the authors that the main reason for the high percentage of return to work may stem from the economic structure in Israel, particularly since 17 % of those who resumed work should have been disqualified on medical grounds. The authors further emphasize the significance of planned rehabilitation as a factor facilitating return to work.

Scandinavia

The results of investigations carried out in Sweden are presented together in Table B (Biörck & Wedelin 1964). The first work considered is based on a group of 85 patients from Malmö (Biörck & Trulson 1957). Their ages were relatively high, ranging from 40 to 89 years with a mean of 65 years. At the onset of the illness only 41 of the men and 5 of the women were capable of working (54 % of the material). 80 % of these were able to resume their previous occupation or some lighter job after an average of 6 weeks of hospital treatment and 5 months of sick-leave. Among the patients aged 40—49 years, half were able to continue their previous work and the other half to assume some lighter occupation, while no one was obliged to abandon work. The authors also report that women made a poorer recovery in all respects than men, even when their work was lighter.

The original material of Malmcrona et al. (1962) consisted of 318 cases with initial myocardial infarction. The average follow-up time was 3 years 5 months. Data were available for 155 of the 195 survivors. The age range of the material was 30—55 years, the mean age 48 years. Of the total of 318 patients 39 % had died by the time for re-examination while about 34 % had regained full working capacity. There were 5 patients who had been

Table B. *Return to work of patients in 3 Swedish cities* (Biörck & Wedelin 1964)

	Number of patients			Age groups (range and mean)	Return to same work	Return to less demanding work	Not working after infarction — working before
	Males	Females	Total		(% of those patients who had been working before infarction)		
Stockholm[1])	149	51	200	40—89 (64)	67 (56)	32 (27) (83)	21 (17)
Göteborg[2])	129	26	155	Up to 55 (48)	82 (53)	49 (32) (87)	19 (13)
Malmö[3])	54	31	85	40—89 (65)	29 (63)	8 (17) (80)	9 (20)

[1]) Biörck and Wedelin 1964
[2]) Malmcrona et al. 1962
[3]) Biörck and Trulson 1957

unable to work even before their infarction. Of the remaining 150 patients 87 % returned to work. Most of them resumed work within one year but 6 % were able to do so only later. Among those returning to work 55 % continued in their old work and 32 % assumed some lighter job. Patients from the higher social classes returned to work more frequently than those with heavier more physical occupations.

The material of Biörck & Wedelin (1964) consisted of 354 survivors, who came from the area of Greater Stockholm, further data being available for 200 of these. Their ages ranged from 36 to 87 years with a mean of 64 years. In each case the myocardial infarction was the last for the patient, so that the figures tabulated are not strictly comparable. The average follow up period was 3.9 years. Of these 200 patients, 120 (60 %) — 113 men and 7 women — had been working at the time of their last infarction, and no less than 16 % of these 120 were continuing in their work despite the fact that they were already receiving old age pension. A total of 83 % returned to some type of work 56 % to their previous occupations and 27 % to some less demanding work. The authors make the interesting observation that all but one of the 19 people (16 %) who were receiving old age pension at the onset of the infarction returned to work 13 of them resumed their old jobs, 5 assumed some lighter occupation. It was also remarkable that 11 of these were doing work that could be defined as moderately active. Of the 99 men with one infarction 50 % returned to the same work as before, 20 % went on to do less demanding work, and 30 % stopped working. Marital status had no effect on return to work. Biörck & Wedelin further assume that sedentary workers (usually synonymous with the middle and upper socio-economic groups) are particularly liable to infarctions, but are at the same time particularly fortunate in their capacity to survive them. Most of them had a new job arranged for them by their employers and less than 10 patients had to resort to official agencies in order to obtain employment.

The Norwegian Knutsen (1960) uses as his material a group of 101 men, 14 % of whom had had two or more infarctions. Their ages ranged from 30 to 67 years, and the follow up periods from 5 to 41 months. There were 86 survivors, divided into three groups according to the strenuousness of their work. Of the 39 patients who had been doing heavy physical work prior to the infarction 44 % regained full working capacity 38 % achieved partial working capacity and the remainder were left permanently incapacitated. For the 30 men who had been doing light physical work, the corresponding figures were 67 %, 20 % and 13 %. The third group consisted of the remaining 17 men who were not doing physical work. The figures for their recovery were 71 %, 18 % and 11 %, respectively. Considering these three groups together 80 % recovered sufficiently to return to some form of work. Two thirds of those capable of working resumed their previous occupations, and were able to manage reasonably well. One third had to assume lighter work or reduce their activity in their former occupation. Knutsen points out that many coronary patients are absent from work too long. Two-thirds of the patients resumed work within six months of the onset of the illness. Another Norwegian account (Lund—Johansen 1965) concerns 196 males with recent myocardial infarction. All were under 70 years of age, and none were receiving old age pensions. Of the 163 patients rehabilitated, 12. (75 %) returned to work, more than 91 of these resuming their previous occupations. The percentages of patients resuming work were 72 %, 71 % and 78 % respectively for the groups of heavy light, and non-manual workers. 58 % of the seamen who suffered infarction were able to return to work. A total of 85 % resumed work in less than 6 months.

Finland

Previously published reports from Finland contain relatively favourable percentages for return to work. Räsänen (1951) discussing the 144 patients for whom data were available out of 250 patients from Helsinki with initial infarction, found no essential differences between the men and the women returning to work. Most of the patients were able to resume work within 3 months of the onset of the infarction, but those with the slowest recovery rate occasionally took up to two years. Most of those who returned to work were under 65 years of age. About 46 % regained full working capacity, 37 % made a partial recovery, and 17 % had to abandon work entirely. These values agree closely with the Scandinavian results presented above.

Iisalo et al. (1958) discuss 370 cases of acute myocardial infarction from Turku. 242 of the patients were re-examined at some time between 6 months and 5 years after discharge. Most of the patients were 40—70 years old. 60.7 % resumed their previous work, while 5.8 %, mostly patients in the older age-groups, took up some lighter occupation.

The distribution of these cases among various occupational groups is examined by Linko (1958). His results are presented in table C. Group I amounting to 21 % of the total consists of professional people, group II of farmers (16 %), group III of supervisors and skilled manual workers (37 %) and group IV of unskilled workers.

The Table C shows clearly that groups II and IV which require greater physical effort (farmers and unskilled workers) had the lowest proportions returning to work. A total of about 32 % were incapacitated, most of them suffering from stenocardia (66 %) or congestive heart failure (13 %) or both (21 %).

Sipila (1966) discusses acute myocardial infarction in a total of 1860 patients from Helsinki aged 29—90 years. The follow-up periods varied from 3 to 18 years. Data on subsequent working capacity were available for 507 persons, 382 men and 125 women. 62.0 % of the survivors assumed jobs similar to their previous occupations, while 17.2 % took up lighter work. Thus 79.2 % of the survivors were still capable of work. There were small differences between the proportions of men and women returning to work. 66.0 % of the men continued in their old jobs, while only 49.6 of the women did so, but 17.0 % of the men and 17.6 % of the women assumed lighter work. Most of the survivors capable of work were under 65 years of age.

Table C. *Return to work after the first myocardial infarction in a material collected from Turku, Finland (Linko 1958, Iisalo et al. 1958). See also the text.*

Occupational group	Proportion (%) returning to work after the specified period following discharge					Not returning to work
	1 mo	3 mo	6 mo	12 mo	over 12 mo	
I Professionals	29	69	87	91	91	9
II Farmers	—	16	48	52	60	40
III Supervisors and skilled manual workers	10	41	63	65	70	30
IV Unskilled workers	—	20	38	40	40	60
Total	11	40	61	66	68	32

Summary of the literature

As can be seen from Table A, the percentages for return to work have in most cases been calculated from a limited number of the survivors, i.e. those for whom there was subsequent data available. Similarly there is often no record of how many patients were fully capable of work at the onset of the infarction. Moreover the size of the original material has not always been stated, nor have the myocardial infarctions always been classified into categories of first attacks and recidives. As the age factor also substantially influences the results, i.e. patients in the older age-groups return to work less frequently and the pensionable age varies from country to country the studies are not strictly comparable. At the same time there is no uniform classification applicable to the occupational groups. Different countries have different economic structures and, consequently different occupational structures. The same can be said of different regions within one country One may be dominantly industrial, another mainly rural. These factors affect the problem under discussion, particularly in the case of those patients who are forced to adopt a less demanding occupation than previously

Some of the studies discuss return to work in relation to the severity of myocardial infarction It should be pointed out, however that the definition of the grades of severity varies greatly in studies from different countries. Thus this aspect also lacks any uniform standardization The influence of the anatomical location of myocardial infarction and the nature of muscular lesion, and also the part played by rehabilitation are further factors on which agreement has not yet been reached.

Finally return to work is also crucially related to the patient's socio-enonomic background and the possibility of obtaining state help The literature reveals certain common features in the studies on return to work, regardless of the approach. First of all, the younger age-groups resume work more frequently than older ones, and generally a higher proportion of men return to work than of women. Most of the studies reviewed discuss return to work on the basis of the known survivors. These usually yield relatively high percentages (50—90 %). The figures are normally higher in the higher socio-economic occupations and in the sedentary groups. Correspondingly return to work is the more difficult the heavier the patient's original occupation has been, with a heavy occupation usually signifying a low socio-economic position

Finland

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V STRUCTURE OF THE MATERIAL

1 *Age and sex distribution*

The youngest patient was aged 28 years, the oldest 92 years. The total of 868 cases included 644 men and 224 women which yields a man-to-woman ratio of 2.9 There were 568 cases under 65 years of age, 453 of them men and 115 women. All those who were able to work at the onset of the illness numbering 360 altogether were under 65 years of age. These cases will be discussed in more detail in the chapter on return to work.

Table 1 shows the age and sex distribution of the total material and the man-to-woman ratios in the different age groups. Table 2 shows the total material classified according to the number of infarctions suffered and the age and sex of the patients. Thus column I contains the cases with recent infarction, column II those with first recidive, and column III all the remaining cases with second or more recidives. The patients with third or fourth recidives were all men and they are included in column III, so that 2 patients with a third recidive are included in the 55—59 year age-group 1 in the 60—64 year group 2 in the 65—69 year group, and 1 in the 75—79 year group The 50—54 year age-group also includes one patient with a fourth recidive and the 70—74 year age group one. Figure 1 is a graphical representation of the data contained in Table 2.

The mean age for the total material was 57 4 years, that for the men was 55.3 years and for the women 64 6 years. The corresponding figures for those with recent infarction were 57 1 55 1 and 62.3 years.

Table 1 *The age and sex distribution of the total material and the male-to-female ratio in the different age groups.*

Age	Male	Female	Total	M:F
—29	1	1	2	1
30—34	12	—	12	
35—39	25	2	27	12.5
40—44	48	3	51	16
45—49	73	4	77	18.2
50—54	82	25	107	3.3
55—59	109	36	145	3.0
60—64	103	44	147	2.3
65—69	83	37	120	2.2
70—74	54	34	88	1.6
75—79	37	24	61	1 5
80—84	13	11	24	1.2
85—89	3	3	6	1
90—	1	—	1	
Total	644	224	868	2.9

Comment

The mean age of the men with recent infarction is about 5 years lower than the corresponding mean age of the women. As can be seen from Table 2, the younger age-groups of the category of recent infarctions contain almost exclusively men. The man to-woman ratio declines fairly sharply after the age of 50 Similar observations have been made in several other studies reporting total man to-woman ratios of 5—1.2 men per 1 woman (Master & Dack 1940 Levine & Rosenbaum 1941 Rathe 1942, Mintz & Katz 1947 Brahme & Ahlberg 1947 Helander 1950, Wållgren 1950 Jacobs 1951 Eckerström 1951 Rasänen 1951 Linden 1952, Cole et al. 1954 Linko

IV MATERIAL AND METHODS

The present material consists of all the 868 undisputable cases of myocardial infarction treated in the Oulu District Hospital during the period 1. 1. 1966—30. 4. 1970.

The following criteria were used

1) Typical ECG finding
   - with or without typical history
   - with or without typical enzymatic finding GOT and/or HBD elevated
   - with or without typical clinical picture usually associated with elevated ESR, leucocyte count, or axillary temperature (the rises not necessarily simultaneous in all these)

   No conclusive evidence of recent myocardial infarction in the ECG but history, clinical picture and elevated enzyme values (or one value elevated) suggest myocardial infarction.

   The LBBB cases which satisfied these criteria were similarly classified into this group.

3) Cases in which there were no ECG or laboratory values available, but the patient's history and the clinical picture suggested myocardial infarction, and obduction later revealed recent cardiac infarction.

Excluded from this material are all cases *in which the history seemed to indicate myocardial infarction*, and the patient died of arrhytmia, but obduction revealed no conclusive proof of myocardial infarction.

The follow-up of the survivors of myocardial infarction was discontinued on 30. 10. 1970, so that the follow-up periods ranged from 6 to 58 months. The socio-economic data and the data concerning working capacity were checked in the district offices of the Sickness Insurance and National Pensions Institute.

The data were processed at the University of Oulu Computer Centre.

The statistical significancies were calculated by the chi-square method and t-test using the *following degrees of significance*: the difference was "almost significant" if the probability of error was less than 5 %, "significant" if less than 1 %, and "highly significant" if less than 0.1 %.

2 *Marital status*

Table 3 shows a comparison of the figures for marital status in the total material and in the age-groups under 65 years. It can be seen first of all, that the proportion of unmarried men is the same in the two groups, 5.7 %, lower than the corresponding figure for unmarried women. The figures for married men are of the same order of magnitude in the two groups, viz. about 90 %, whereas the figures for married women differ appreciably The proportion of married women in the total material is about 49 %, and in the group aged under 65 years about 63 %. The figures for female widows show the opposite trend the total material contains 37 % widows, the second group about 26 %. This provides indirect support for the observation already made when discussing the age structure, viz. that women suffer infarction at a more advanced age than men do.

Comment

According to Biörck et al. (1958) and Sievers (1964) in Sweden, married men were more liable to suffer infarction than either men of any other marital status regardless of age, or married women of 70 and more. The difference was statistically significant amongst the men of 50 or more, and almost significant for the oldest class of women Gorbatow (1961) noted in his work that married women had a higher frequency of infarctions than was to be expected on the basis of the population structure of Helsinki, but this tendency appeared less marked as age increased. The statistics necessary for a comparison of this kind were not available to the present author Nothing can therefore be said about the significance of these findings. The present work only reveals a noticeable tendency towards a higher-than average incidence of myocardial infarctions among married men in both the total material and the group under 65 years of age.

When discussing the etiology of coronary disease, authors have also paid attention to environmental stress factors. Thus Skyring et al. (1963) reported proportionally more coronary deaths among subjects under 45 years old who had been married several times than amongst controls of the same age. Bruhn et al (1968) similarly noted that coronary disease were more frequent among both male and female subjects who had more marital problems than the controls.

3 *Place of residence*

Table 4 indicates the places of residence for the total material and for the age-groups under 65 It can be seen that about 45 % of

Table 3 *Marital status. Comparison of the total material and those under 65 years old*

Marital status	Total material			Patients under 65 years old.		
	M (%)	F (%)	Total (%)	M (%)	F (%)	Total (%)
Unmarried	37 (5.7)	28 (12.5)	65 (7.5)	26 (5.7)	10 (8.7)	36 (6.3)
Married	555 (86.2)	110 (49.1)	665 (76.8)	411 (90.7)	72 (62.6)	483 (85.0)
Widowed	38 (5.9)	83 (37.0)	121 (13.9)	7 (1.6)	30 (26.1)	37 (6.5)
Divorced	4 (0.6)	2 (0.9)	6 (0.7)	4 (0.9)	2 (1.7)	6 (1.1)
Not known	10 (1.6)	1 (0.5)	11 (1.1)	5 (1.1)	1 (0.9)	6 (1.1)
Total	644 (100.0)	224 (100.0)	868 (100.0)	453 (100.0)	115 (100.0)	568 (100.0)

Table 4 *Place of residence*

Place of residence	Total material			Patients under 65 years old.		
	M (%)	F (%)	Total (%)	M (%)	F (%)	Total (%)
Town of Oulu	285 (44.3)	112 (50.0)	397 (45.8)	198 (43.8)	56 (48.7)	254 (44.7)
Elsewhere in the Province of Oulu	341 (52.9)	108 (48.2)	449 (51.7)	239 (52.7)	57 (49.6)	296 (52.1)
Province of Lapland	7 (1.1)	2 (0.9)	9 (1.0)	6 (1.3)	—	6 (1.1)
Elsewhere in Finland	11 (1.7)	2 (0.9)	13 (1.5)	10 (2.2)	2 (1.7)	12 (2.1)
Total	644 (100.0)	224 (100.0)	868 (100.0)	453 (100.0)	115 (100.0)	568 (100.0)

the patients in each group lived in the town of Oulu (pop 88.000) Elsewhere in the Province of Oulu refers to the surrounding rural district within a radius of about 60 km. (pop 70 000) There is no coronary register for the area, but the Oulu District Hospital is the only hospital serving this area which receives acute cases, such as myocardial infarctions, requiring hospital treatment. There are other hospitals in Oulu, the Oulu City Hospital and the Oulun Diakonissalaitos hospital, but these do not function as reception hospitals for acute diseases of this kind.

The distribution of place of residence among the patients who were capable of work at the onset of the illness, all under 65 years of age, is presented in Table 11 It can already be said however that about 48 % of the men came from Oulu and a similar proportion from the surrounding rural district, whereas as many as 61 % of the women who were able to work at the onset of the illness came from the town of Oulu, and only 37 % from the country

Comment

Place of residence and nature of occupation are two interesting factors in the studies of coronary disease. Various statistical comparisons have been made. From Iceland Sigurjonson (1969) for example, reported that the figure for mortality from ischemic heart disease per 100 000 inhabitants was 175 4 in Reykjavik but only 78.2 in the country The age structure of the population, migration local conditions as determinants of the nature of occupation, and habits and customs, which are often closely associated with the above two factors, are questions whose real significance is almost impossible to estimate.

4 *Occupation*

Table 5 shows the distribution of occupations in the total material, in the portion aged less than 65 and in the group capable of work at the onset of the illness. The first occupational group unskilled workers, consists of all the workers who have not been trained for their work, such as unskilled manual workers, various auxiliaries, and cleaners, as well as the spouses of such workers. The second group small farmers, includes the owners of small farms who are forced to adapt secondary sources of livelihood such as lumbering. This group mainly consists of lumberjacks. The subjects classified into this group must have also reported themselves as working as small farmers or

Table 5. *The distribution of occupations as percentages in the total material, in the portion aged under 65 and in the group able to work at the onset of myocardial infarction.*

Occupation	Total material			Patients under 65 years old			Able to work at the onset of M.I.		
	M	F	Total	M	F	Total	M	F	Total
Unskilled labourers	21.4	25.0	22.3	21.6	26.9	22.7	20.1	25.8	21.1
Small farmers, lumberjacks	7.9	8.5	8.1	8.2	7.8	8.1	7.4	6.5	7.2
Farmers	24.1	26.8	24.8	19.9	22.6	20.5	16.1	17.7	16.4
Skilled employees in commerce and communications	15.4	8.5	13.6	17.2	9.5	15.7	16.4	11.3	15.5
Skilled industrial workers	8.4	2.7	6.9	11.0	3.5	9.5	14.1	6.5	12.8
Supervisors, foremen	10.5	0.9	8.1	11.5	0.9	9.3	12.1	0	10.0
Small-scale businessmen	2.5	1.3	2.2	2.2	2.6	2.3	2.0	4.8	2.5
Civil servants	3.1	2.2	2.9	3.3	0.9	2.8	4.4	1.6	3.9
Managerial position, academic degree	6.1	1.8	4.9	5.1	0.9	4.2	7.4	1.6	6.1
Housewives	0	22.3	6.2	0	24.4	4.9	0	24.2	4.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number of cases	644	224	868	453	115	568	298	62	360

lumberjacks, and these cases have been checked in the Sickness Insurance files, for the difference between this group and the next, which consists of farmers whose main source of livelihood is farming, is often very small. Most of the farmers in this area do occasional lumbering as well. The women in the third group do all types of heavy farm work, such as tending the cattle, and their day's work is not limited to a certain number of hours as it is in factories. Most of the work done by the wives of unskilled workers and small farmers is naturally similar to this, but they have been classified according to their husband's occupation.

The next group consists of skilled workers in commerce and communication, including shop assistants, salesmen and saleswomen, post office workers, bus and lorry drivers, etc., most of whom live in the centres of population. The skilled industrial workers are trained and well organized, and are similarly concentrated in centres of population.

A separate group is set aside for supervisors, irrespective of the field they are working in. Most of them are, in fact, employed in commerce and industry. The proportion of forestry supervisors in the material is about the same as that of any other supervisory group. Supervisors are often in a difficult position: they must convey the orders and instructions of the management to the employees, and require adequate working performance to satisfy the employer.

Small scale businessmen, small private enterprises and self-employed workers nowadays often succumb to the large co-operative firms and companies, and frequently go bankrupt, unless they are specialized in some particular field. Small shops are disappearing in the face of proliferating supermarkets. The owners of such enterprises both in the country and in the towns experience continuous stress due to loss of customers and increasing debts on the one hand and the pressure exercised by the large firms on the other. The mental stress on this group is probably even greater than that on the supervisors. This assumption is supported by *all* the interviews conducted with these patients, and the experience of sickness insurance officials concerning the income structure of these people and their pro-

neness to myocardial infarction. A person running a small commercial enterprise is economically insecure in comparison with a wage-earning skilled worker

The group of minor civil servants consists of people mainly doing sedentary office work. This group also includes policemen and primary school teachers, who represent the higher socio-economic strata within the group

The term managerial position refers to people who are responsible for managing a firm, office, institution, etc. Most of these have an academic degree, being thus the best educated and holding the highest positions in the socio-economic hierarchy

Housewives are treated as a separate group As used in the present context, the term refers to women who do not work for wages, but spend their days mainly at home, and whose husbands mostly belong to the socio-economic group of the managers and supervisors. The work they do is light. The remainder of the women are classified according to the occupation of their husbands.

The distributions of occupations were almost identical in the total material, the group under 65 and the group of those capable of work at the onset of the illness. The largest portion in each of these groups consisted of unskilled and untrained people, most of whom lack any permanent employment and lose their jobs as soon as the general employment situation deteriorates. More than a fifth of the present material consisted of such people.

The age factor appears indirectly in the column for the total material The group aged less than 65 years forms an intermediary stage, and those capable of work at the onset of infarction the youngest group, constituting one part of all those less than 65 years old. There is an interesting tendency visible in the percentage distributions of skilled industrial workers and supervisors as one moves from the youngest group on the right to the oldest group on the left in Table 5 The relative proportion of such people is clearly smaller in the older part of the material than in the younger Whether this is partly due to the stress of the work remains to be guessed at, but the general tendency in indisputable. No such observation could be made in regard to the other occupations. On the contrary the relative proportion of farmers increases in the older groups.

Master & Jaffe (1952) compared the occupational structures of their material of myocardial infarctions with the structure of the population at large in the same area, and concluded that occupation and social status do not affect the incidence of coronary occlusion Adelson & Hoffman (1961) observed that sudden deaths from coronary disease occur among people with all types of occupation No differences in the incidence of myocardial infarction between the different occupational groups could be found in a group of middle-aged men from Oslo (Westlund 1961) Similar findings have also been made in some prospective epidemiological works. No differences related to educational background could be shown after the fourth year in the Framingham study (Dawber et al. 1957) but after the sixth follow-up year the data showed a significant trend in the incidence of coronary heart disease in relation to educational level, the incidence being less at the higher levels, particularly among young men (Dawber et al 1959) In a Chigaco utility company the incidence rates of coronary heart disease were similar in all sociological subgroups (Stamler et al. 1960) Similarly the investigation of an industrial population over a period of over 4 years revealed no association between the incidence of myo-infarction and type of job or physical activity outside work hours (Paul et al. 1963).

An inverse relationship between habitual physical activity and proneness to ischaemic or coronary heart disease has been suggested, but not clearly established by studies of bus

drivers, conductors, post office workers (Morris et al. 1953 a, b and 1966) and railway employees (Taylor et al. 1962) Similar results were obtained by Brown et al. (1957) and Sarvotham & Berry (1968), and the same phenomenon was noted in the necropsy survey of Morris & Crawford (1958) Yater et al. (1948 a) in their study of a group of soldiers under 38 also found those in sedentary occupations to be more susceptible to myocardial infarction.

Statistics have shown mortality from coronary disease in USA to be higher in the lower social classes than elsewhere (Lilienfeld 1956, Lew 1957) Similarly a larger proportion of infarction cases occurred among the lower-class workers in an industrial establishment than among the managerial staff (Pell & D Alonzo 1958) The opposite was found in England (Morris 1956) Men aged 60—70 years in the upper social classes were found to be more prone to infarctions than those in the lower classes (Brown 1962)

Research has also revealed the increased predisposition of the general practitioner to coronary artery disease (Morris e al. 1952) The incidence of such disease was about twice as high as among medical specialists and other population groups in England. A similar phenomenon was found in an American study which showed coronary disease to be more frequent among those experiencing stress in various occupations (Russek & Zohman 1958) and to be three times more prevalent among general practitioners than among dermatologists and pathologists. Anaesthetists considered to be second to general practitioners in the stress suffered, came close to them, and in their 50's and 60's actually exceeded them, in the frequency with which coronary disease was reported (Russek 1960) A similar situation was encountered in a later study conducted on American physicians, dentists and lawyers (Russek 1962). Hinkle et al. (1968) Hinkle (1969) and Shekelle (1969) found myocardial infarction to be more common among men who had not attended college than among those with a college education.

In Scandinavia, the work of Biörck et al. (1958) showed myocardial infarction to be substantially more common amongst employers than amongst the civil servants and workers. The occupational distribution of male patients under 70 showed a higher proportion of executives and considerably fewer workers than was to be expected on the basis of the occupational distribution of Norwegian urban populations as studied from Oslo (Oslo material 1956) The Norwegians Sivertssen et al. (1957) similarly noted that there was a preponderance of patients with professional occupations and relatively few patients engaged in heavy manual labour

In his work on coronary deaths, Brown (1962) observed that farmers and other agricultural workers had the lowest mortality rate, whether from all causes or from coronary thrombosis, and clerical workers the highest. Brown assumes the reason for this to be the lack of haste, the protective effect of physical activity and regular habits.

Finland

Ikkala & Kaipainen (1957) discuss a small group of young patients with myocardial infarction representing different occupations. The myocardial infarction cases from the Oulu District Hosp tal described and published by Vartio (1960) showed an occupational structure similar to that of the remaining patients in the hospital. The patients of high social status in Gorbatow s account (1961) had a higher incidence of myocardial infarction than could have been expected on the basis of the total distribution of the population of Helsinki. Similarly the percentage recorded for the lowest social class was smaller than would have been suggested by the population structure. The women in the lowest social class suffered infarctions more

frequently than those in the other classes. Kasanen et al. (1963) compare the socio-economic and psychic stress undergone by a set of 100 patients aged 30—60 years with that undergone by a similar control group This work similarly revealed the incidence of myocardial infarction to be higher in responsible jobs and sedentary occupations.

Finland is one of the standardized research areas in an extensive international programme designed to elucidate the etiology of coronary disease (Keys 1970) It has already been shown that in one municipality in eastern Finland lumberjacks had significantly fewer pathological changes in ECG indicative of past myocardial infarction and myocardial ischaemia than any other male group (Karvonen et al. 1961) An epidemiological investigation comparing the eastern and western parts of Finland over a period of 5 years revealed the prevalence of coronary diseases standardized for age to be 5.5 % in the east, whereas the corresponding figure for the west was only 1.6 %, a remarkable difference. In each area about 70 % of the men aged 40—60 years worked in farming or lumbering. 71 % in the east and 77 % in the west performed very heavy physical work, 11 % and 9 % respectively performed very light work. The incidence of CHD was related to physical activity or the lack of it exept in Western Finland, where CHD incidence was excessive among moderately active men, with both sedentary and extremely active men being less prone to the disease. (Karvonen et al. 1970)

Table 5 A shows a comparison of the occupational structures of cases of myocardial infarction recorded at the Oulu District Hospital and the University Central Hospital of Turku. The material from Turku was collected during the period 1966—1970 (Vuopala et al.)

It can be seen that the group of unskilled workers is clearly larger in the north, the mean percentages being 21 1 % and 15 6 %. There are hardly any small farmers or lumberjacks amongst the Turku cases, and although they are not very numerous in the present material either they do constitute 7.2 % of the cases as opposed to 0 9 % in Turku. The proportion of farmers shows the reverse relationship which is naturally explicable by the agricultural structure in the south of Finland. Skilled industrial workers constitute the largest group in Turku, being proportionally more numerous than in Oulu.

Tabl. 5 A *The oc pational distribution of the patient apable f work at the onset f myocardial infarction xpr sed as percentages. The materials ollected from Oulu and Tu ku ompared*

Occupation	Males		Females		Total	
	Oulu	Turk	Oul	Turk	Oulu	Turk
U skilled labourers	20.1	15.5	25.8	16.3	21 1	15.6
Small farmers lumberjacks	7 4	1.0	6.5	—	7.2	0.9
Farmers	16.1	8.6	17.7	9.2	16.4	8.7
Skilled employees in commerce and communications	16.4	13.8	11.3	10.2	15.5	13.2
Skilled industrial workers	14 1	25 9	6.5	6.1	12.8	22.6
Supervisors, foremen	12.1	11.3	—	2 0	10.0	9.7
Small-scale businessmen	2.0	8.2	4.8	7.2	2.5	8.0
Civil servants	4 4	7.3	1.6	7.2	3 9	7 4
Managerial position, academic degree	7 4	8.4	1.6	5 1	6.4	7.5
Housewives	—	—	24.2	31.8	4.2	6.
Total	100	100.0	100.0	100.0	100 0	100.0
Number of cases	298	485	62	98	360	583

The relative proportion of supervisors is about the same in the two places (10 %) The number of patients with other occupations, such as self-employed people, managers, minor civil servants, and housewives is greater in Turku. These distributions are representative of the economic structures of each region (Statistical Yearbook of Finland 1968), so that, roughly speaking, no particular occupation can be pointed out as engendering a predisposition to myocardial infarction.

3 *Economic status at the onset of myocardial infarction*

Table 6 shows the economic status of the cases studied at the onset of myocardial infarction from males and females, as well as the total figures and the male-to-female ratio

Comment

The literature contains only a few accounts of the proportion of patients able to work, on pension, or on sick leave at the onset of the illness (Biörck & Trulson 1957 Malmcrona et al. 1962 Biörck & Wedelin 1964) The figures presented here are considerably higher in the category of pensioners, a finding further emphasized by the fact that the pensionable age is 2 years higher in Sweden than in Finland. It can be seen that one third of the patients suffering from myocardial infarction were receiving old age pensions at the time, and one fifth of those under 65 years old were, for some reason or another receiving invalid pensions. Th man-to-woman ratio reveals the effect of the age factor once more the difference between the sexes diminishes in the higher age-groups.

For the sake of comparison, the corresponding distribution in the previously mentioned data from Turku is presented in Table 6 A. Here the patients receiving invalid pensions and those on sick leave are combined.

Table 6 A *Comparison of economic status at the onset of myocardial infarction in the materials collected from Oulu and Turku. The figures given are percentages.*

Economic status	Oul	Turk
Fully able to work	41.5	37.3
Sick leave + sickness pension	23.6	14 9
Employment pension	0.3	0.9
Old-age pension	34.6	46.9
Total /	100.0	100.0
Number of cases	868	1566

The proportion of those capable of work is almost identical in the two groups about 40 %. The proportion of people under 65 receiving invalid pensions or on sick leave, however was more than one and a half times greater in the Oulu material. In each case about 5 % were on sick-leave.

Table 6. *Economic status at the onset of myocardial infarction in the total material.*

Activity	Males (%)	Females (%)	(Total (%)	M F
Fully capable of work	298 (46.3)	62 (27.7)	360 (41.5)	4.8
Employment pension	2 (0 3)	1 (0.4)	3 (0.3)	2
Sick-leave	35 (5 4)	11 (4 9)	46 (5.3)	3.2
Sickness pension, under 65	118 (18.3)	41 (18.3)	159 (18.3)	2.7
Old-age pension, 65 or over	191 (29.7)	109 (48.7)	300 (34.6)	1.8
Total	644 (100.0)	224 (100 0)	868 (100.0)	2.9

frequently than those in the other classes. Kasanen et al. (1963) compare the socio-economic and psychic stress undergone by a set of 100 patients aged 30—60 years with that undergone by a similar control group This work similarly revealed the incidence of myocardial infarction to be higher in responsible jobs and sedentary occupations.

Finland is one of the standardized research areas in an extensive international program me designed to elucidate the etiology of coronary disease (Keys 1970) It has already been shown that in one municipality in eastern Finland lumberjacks had significantly fewer pathological changes in ECG indicative of past myocardial infarction and myocardial ischaemia than any other male group (Kar vonen et al 1961) An epidemiological inves tigation comparing the eastern and western parts of Finland over a period of 5 years revealed the prevalence of coronary diseases standardized for age to be 5.5 % in the east, whereas the corresponding figure for the west was only 1.6 %, a remarkable difference. In each area about 70 % of the men aged 40—60 years worked in farming or lumbering. 71 % in the east and 77 % in the west performed very heavy physical work, 11 % and 9 % respectively performed very light work. The incidence of CHD was related to physical activity or the lack of it except in Western Finland, where CHD incidence was excessive among moderately active men, with both se dentary and extremely active men being less prone to the disease. (Karvonen et al. 1970)

Table 5 A shows a comparison of the occupational structures of cases of myocardial in farction recorded at the Oulu District Hospital and the University Central Hospital of Turku. The material from Turku was col lected during the period 1966—1970 (Vuopala et al.)

It can be seen that the group of unskilled workers is clearly larger in the north the mean percentages being 21 1 % and 15.6 %. There are hardly any small farmers or lumberjacks amongst the Turku cases, and al though they are not very numerous in the present material either they do constitute 7.2 % of the cases as opposed to 0.9 % in Tur ku. The proportion of farmers shows the re verse relationship which is naturally explicable by the agricultural structure in the south of Finland. Skilled industrial workers constitute the largest group in Turku, being proportionally more numerous than in Oulu.

Table 5 A. *The oc pational distribution f the patie ts capable f work at the onset f myocardial infarc tion xpressed as percentages The material ollected from Oulu and Turku ompared*

Occupation	Males		Females		Total	
	Oulu	Turk	Oulu	Turku	Oul	Turk
Unsk lled labourers	20 1	15.5	23.8	16.3	21 1	15.6
Small farmers, lumberjack	7 4	1.0	6.5	—	7.2	0.9
Farmers	16.1	8.6	17.7	9.2	16.4	8.7
Skilled employ es in commerce nd communications	16.4	13.8	11.3	10.2	15.3	13.2
Skilled industrial orkers	14 1	25 9	6.5	6.1	12.8	22.6
Supervisors, foremen	12.1	11.3	—	2.0	10.0	9.7
Small-scale businessmen	2.0	8.2	4.8	7.2	2.5	8.0
Ci il servants	4 4	7.3	1.6	7.2	3 9	7 4
Managerial position, academic degree	7 4	8.4	1.6	3 1	6.4	7.5
Housewi es	—	—	24.2	38.8	4.2	6.
Total	100..	100.0	100.0	100.0	100.0	100.0
Number of cases	298	485	62	98	360	583

Table 8 *Economic status f the total material at the onset f myocardial infarction ac ording to the number f infarctions suffered.*

Activity	Recent infarction				First recdi				Second or more recid es				
	M	F	Total	M:F	M	F	Total	M:F	M	F	Total	M:F	Total
Patients under 65 capable of work	262 (54.7)	57 (31.3)	319 (48.3)	4.5	32 (26.2)	4 (12.5)	36 (23.4)	8	4 (9.1)	1 (11.1)	5 (9.4)	4	360 (41.5)
Sick-lea under 65	15 (3.1)	8 (4.4)	23 (3.5)	1.9	18 (14.8)	2 (6.3)	20 (13.0)	9	2 (4.5)	1 (11.1)	3 (5.7)	2	46 (5.3)
Employment pension nder 65	1 (0.2)	—	1 (0.1)		—	1 (3.1)	1 (0.6)		1 (2.3)	—	1 (1.9)		3 (0.3)
Sickness pension nder 65	56 (11.7)	6 (14.3)	82 (12.4)	2.2	37 (30.3)	13 (40.6)	50 (32.5)	2.8	25 (56.8)	2 (22.2)	27 (50.9)		159 (18.3)
Old-age pension over 65	145 (30.3)	91 (50.0)	236 (35.7)	1.6	35 (28.7)	12 (37.5)	47 (30.5)	2.9	12 (27.3)	5 (55.6)	17 (32.1)	2.4	300 (34.6)
Total no of cases Total /	479 (100.0)	182 (100.0)	661 (100.0)	2.6	122 (100.0)	32 (100.0)	154 (100.0)	3.8	44 (100.0)	9 (100.0)	53 (100.0)	5.9	868 (100.0)

creases in the present material the figure was 48.3 % in the group of recent infarctions, 23.4 % in the group of first recidives and 9.4 % in the group of second or more recidives.

About one third of each group received old age pensions. The proportion of people under 65 receiving invalid pensions increases with the number of infarctions suffered, the figures for the different groups being 12.4 %, 32.5 % and 50.9 %.

The male-to-female ratio increases from 2.6 in the group of recent infarctions, to 3.8 in the group of first recidives, and 5.9 in the group of second or more recidives.

Comment

In the Swedish material collected during the years 1950—1954 by Biörck et al. (1958) 30.4 % of the 940 men had already retired by the time they suffered their first infarction. In the present material 30.3 % of the men were receiving old age pensions when their first infarction occurred. The total figure for this material is higher, however, for we must include the patients under 65 who had retired because of ill health (11.7 %) or were receiving employment pensions (0.2 %). Thus 42.2 % of the 479 men in the present material had already retired at the time of their first infarction. We should also add that the pensionable age is 65 in Finland but 67 in Sweden which makes the difference even more pronounced.

Wahlberg (1963) who carried out his investigation in Seraphimer Hospital in Stockholm, noted that the patients with one, two, and three myocardial infarctions taken as separate groups had the following male-to-female ratios: 2.1, 4.2 and 8.0, respectively. The corresponding figures in the present material were 2.6, 3.8 and 5.9, showing the same tendency.

VI RESUMPTION OF WORK AND FACTORS INFLUENCING IT

1 Effect of age and sex distribution

Table 9 shows the proportion resuming work in the different age and sex groups according to the number of infarctions suffered. Since the cases of first and second or more recidives were few in number no percentages have been calculated for these groups. The calculations cover the 360 patients who were capable of work at the onset of the infarction, 126 of whom recovered enough to resume work. The symbol A denotes those members of each group capable of work, B those resuming work. In each age-group therefore, the percentage return to work can be expressed as B.A %.

In the group of recent infarctions, the percentage of those returning to work declines as one moves from the lower age-groups to the higher ones. In the female group aged 55—59 years the percentage is quite low 9.5 %, while none of the women who had had two or more infarctions returned to work. After the first infarction 34 8 % of the total returned to work, the figures for men and women being 36 6 % and 26.3 % respectively

Table 9 *Effect f ge and sex distribution on return t work ac ding to the number f infarction suffered A = the number f cases capabl f work at the onset f M.I B = the number f ases recovered t resume work. The percentag f B A is pressed in parenthesis*

Age group		Recent infarction M	Recent infarction F	Recent infarction Total	First recidi M	First recidi F	First recidi Total	Second or more recidives M	Second or more recidives F	Second or more recidives Total
25—29	A	1	1	2						
	B	1 (100.0 /)	1 (100.0 /)	2 (100.0 /)						
30—34	A	8		8	1		1	1		1
	B	4 (50.0 /)		4 (50.0 /)	0		0	0		0
35—39	A	19		19	2	1	3			
	B	10 (52.7 /)		10 (52.7))	2	0	2			
40—44	A	39		39	1	1	2			
	B	18 (46.2 /)		18 (46.2 /)	0	0	0			
45—49	A	45	1	46	8		8	1		1
	B	17 (37 8 /)	1 (100 0 /)	18 (39 1 /)	4		4	1		1
50—54	A	50	13	63	5		5	1	1	2
	B	22 (44.0 /)	5 (38.5 /)	27 (42.8 /)	1		1	0	0	0
55—59	A	54	21	75	6	2	8	1		1
	B	16 (29.6 /)	2 (9.5 /)	18 (24.0 /)	4	0	4	0		0
60—64	A	46	21	67	9		9			
	B	8 (17 4 /)	6 (28.6 /)	14 (20.9 /)	3		3			
Total	A	262	57	319	32	4	36	4	1	5
	B	96 (36.6 /)	15 (26.3 /)	111 (34.8 /)	14	0	14	1	0	1

Comment

The percentage of return to work decreases as age increases (Cooksey 1935 Master & Dack 1940 Levine & Rosenbaum 1941 Weinblatt et al. 1966)

It is reported that sex had no effect on return to work in any occupational group for almost as many women resumed their former activities as did men (51 % as against 54 % Master & Dack 1940) Some authors, however hold the opposite view Levine & Rosenbaum (1941) for example, noted that 79 % of the men in their study were able to return to full or partial activity whereas only 51 % of the women recovered to that extent. After recovery from an initial myocardial infarction, women tend to live for a shorter time, suffer more congestive failure, and be less often able to return to useful activity Similar observations of the poorer recovery rate among women have been made by Biörck & Trulson (1957), Biörck (1964), Lund—Johansen (1965) Sipila (1966) and Kellermann et al (1968). One natural explanation for this is the fact that women usually suffer myocardial infarction 5—7 years later than men do, a finding which has been discussed in association with the age structure of the cases studied. On the average, this age more or less coincides with the established pensionable ages of the different countries.

2 *Marital status in relation to resumption of work*

Table 10 shows percentage return to work in relation to marital status Comparison with the corresponding categories in Table 3 shows that there are no essential differences among those capable of work at the onset of the infarction or among those aged under 65

3 *Effect of place of residence on resumption of work*

If the distribution of places of residence among those capable of work at the onset of the infarction is compared with the corresponding distribution for those under 65 as presented in Table 4 which is in turn very similar to that for the total material, small differences can now be seen. In Table 11 the proportion of urban dwellers is increased. For men the proportion is almost exactly the

Table 1 *Marital status in relation to return to work.*

Marital status	Those capable of work at the onset of infarction (A)			Those returning to work (B)			B/A in per cent		
	M (%)	F (%)	Total (%)	M (%)	F (%)	Total (%)	M	F	Total
Unmarried	18 (6)	8 (12.9)	6 (7 5)	6 (5 4)	3 (20.0)	9 (7 1)	33.3	37.5	34.6
Married	272 (91 4)	6 (58 1)	348 (85.2)	100 (90.1)	10 (66.7)	110 (87.3)	36 8	27.8	35.7
Widowed	3 (1)	15 (24 2)	18 (5.0)	3 (2.7)	2 (13.3)	5 (4.0)	100.0	13.3	27.8
Divorced	4 (1 3)	2 (3 2)	6 (1.7)	2 (1.8)	—	2 (1.6)	50 0	0	33.3
No data available	1 (3)	1 (1 6)	2 (0.6)	—	—	—			
Total no. of cases Total	298 (100)	62 (100.0)	360 (100.0)	111 (100.0)	15 (100.0)	126 (100.0)			

Table 11 *Effect of place f residenc on return t work*

Place f residence	Those capable f work at the onset of infarction (A)			Those returning to work (B)			B/A in per cents		
	M (%)	F (%)	Total (%)	M (%)	F (%)	Total (%)	M	F	Total
Town f Oulu	144 (48.4)	38 (61.3)	182 (50.6)	72 (64.9)	12 (80.0)	84 (66.6)	50.0	31.6	46.1
Elsewhere in the Province of Oulu	145 (48.6)	23 (37 1)	168 (46 6)	34 (30 6)	3 (20.0)	37 (29 4)	23.4	13.0	22.0
Province of Lapland	2 (0.7)	—	2 (0.6)	1 (0.9)	—	1 (0.8)	50.0	—	50.0
Elsewhere in Finland	7 (2.3)	1 (1.6)	8 (2.2)	4 (3.6)	—	4 (3.2)	57.2	—	50.0
Total umber f cases / Total /	298 (100.0)	62 (100.0)	360 (100.0)	111 (100 0)	15 (100.0)	126 (100.0)			

same 48 4 % and 48.6 % respectively but in the case of women there is a clear difference. While in the group aged under 65 the proportion of women was about 49 % in both urban and rural districts, the group of the patients capable of work at the onset of the illness had 61.3 % of its women from the town and only 37 1 % from the country By comparing the figures for those returning to work, it can be seen that urban dwellers on the whole return to work more frequently than rural dwellers. The difference is large 46 1 % and 22 %. The difference between the sexes is even more pronounced: men are in a more favourable position, 50 % returning to work as compared to 31.6 % of the women. The corresponding figures for the rural group are 23 4 % and 13 %, again showing a substantial difference. The same figures reveal very clear differences in rate of resumption of work between urban and rural men and, correspondingly between urban and rural women. This shows undisputably that both men and women in the country have less opportunities to resume work than their counterparts in urban areas. This is naturally due to the considerable differences in economic structure. The rural occupations are almost exclusively restricted to heavy physical work, whereas towns can offer a large variety of jobs.

For the sake of comparison, Table 11 A shows the sex distribution of those returning to work in Oulu and Turku. The total percentages and those for men vary little between the two cities. About half of the urban men returned to work in both Turku and Oulu, whereas only about one fifth of the men living in the country do so The total percentages show that slightly less than half of the urban people and about one fifth of the rural people return to work both in northern Finland and in the south. These differences are highly significant. The situation is otherwise as regards women 31.6 % of the urban women return to work in Oulu and 45 % in Turku, the corresponding figures for the rural

Table 11 A. *Effect f place f residence on return to work. Comparison of the Oulu and T ku materials by the sexes. The percentages refer to those returning to work.*

Place of residence	Oulu M	Oulu F	Oulu Total	Turku M	Turku F	Turku Total
Urban area	50.0	31 6	46.1	48.4	45.0	47.8
Rural area	23 4	13.0	22.0	20.5	31.6	22.2

areas being 13.0 % and 31.6 %. The differences are again highly significant. It can be concluded that women in the north of Finland return to work less frequently than women in the south, no matter whether they live in the town or in the country. The situation is particularly unfavourable for women in the rural districts of northern Finland, only 13 % of whom resume work.

One reason for the deplorable position of women in the north is the great difference between the urban and the rural areas. The border line between the urban area of Turku and the surrounding rural district is diffuse compared with that prevailing in Oulu. For this reason, the urban economic structure is not so much different from the rural one in Turku as it is in Oulu. This has already been noted in Table 3 A, which shows the occupational distributions of those capable of work at the onset of infarction in Oulu and Turku.

4 Effect of occupation on resumption of work

Table 12 shows the proportions of those capable of work at the onset of the infarction and the percentage returning to work classified by occupational groups. The highest percentage returning to work (73.9 %) was recorded in the managerial group mostly comprising people with academic degrees. About half of the housewives, supervisors, and skilled industrial workers resumed work. Among the skilled employees in commerce and industry and the minor civil servants, the proportion returning to work was over 40 %. One out of three private self-employed people resumes work. More than a fifth of the unskilled workers are able to resume work, whereas only about one tenth (11.9 %) of the farmers are able to do so. The situation is worst of all in the case of small farmers and lumberjacks, of whom only 7.7 % are capable of returning to work. The last three of the occupational groups enumerated are the hardest physically and the differences between these three and the other groups are statistically highly significant, as are also those between the managerial group and the others.

The above applies to the figures calculated for men returning to work; those for women are too small to allow the calculation of de-

Table 1[illegible] *Return to work in the occupational categories*

Occupation	Those capable of work at the onset of infarction (A)			Those returning to work (B)			B/A in per cents		
	M	F	Total	M	F	Total	M	F	Total
Unskilled labourers	60	16	76	16	1	17	26.7	6.3	22.4
Small farmers, lumberjacks	22	4	26	2	0	2	9.1	0	7.7
Farmers	48	11	59	6	1	7	12.5	9.1	11.9
Skilled employees in commerce and communications	49	7	56	24	1	25	49.0	14.6	44.6
Skilled industrial workers	4[illegible]	4	46	20	2	22	47.6	50.0	47.8
Supervisors, foremen	36	—	36	19	—	19	52.8	—	52.8
Small-scale businessmen	6	3	9	2	1	3	33.3	33.3	33.3
Civil servants	13	1	14	6	0	6	46.2	0	42.8
Managerial, academic degree	[illegible]2	1	23	16	1	17	72.8	100.0	73.9
Housewives	—	15	15	—	8	8	—	53.4	53.4
Total	[illegible]98	62	360	111	15	126	37.3	24.2	35.0

grees of significance. The same tendency however is visible in the female material. It is worth noting that the present material contained only three men who were able to take a less demanding job after recovery. They all worked for the state railways. No opportunity of changing jobs existed elsewhere.

Table 13 shows the number of those capable of work at the onset of the infarction (A) and those returning to work (B) in the different age-groups and occupational categories. The numerical values being so low no percentages have been calculated. The general tendency is that the proportion returning to work decreases in each occupational category as age increases. This is particularly obvious in the heaviest occupations, farmers, lumberjacks and unskilled workers. The differences between the age-groups are not so pronounced in the categories of managers and skilled workers.

Comment

In Table 13 A the corresponding percentages from Oulu and Turku are compared. The unskilled workers, small farmers and farmers, who constitute the groups performing the heaviest physical work, return to work much less frequently than people in the other categories. The figures for farmers are almost identical (11.9 % and 10.0 %). None of the few small farmers from the rural outskirts of Turku returned to work, nor did any of the women in the small farmer category from Oulu, while the percentage for men in the latter area was extremely low (7.7 %).

Table 13. *Age and occupation in relation to return to work. A = those capable of work at the onset of infarction. B = those returning to work.*

Age group	Occupation	Unskilled labourers	Small farmers, lumberjacks	Farmers	Skilled employees in commerce and communications	Skilled industrial workers	Supervisors, foremen	Small-scale businessmen	Civil servants	Managerial, academic degree	Housewives	Total
25—29	A	2	0	0	0	0	0	0	0	0	0	2
	B	1	0	0	0	0	0	0	0	0	0	1
30—34	A	0	0	1	5	0	1	0	2	1	0	10
	B	0	0	0	1	0	1	0	1	1	0	4
35—39	A	4	2	3	1	6	4	1	0	1	0	22
	B	1	1	0	1	1	4	0	0	1	0	12
40—44	A	9	5	4	5	8	3	1	2	3	1	41
	B	2	0	0	3	6	1	1	2	3	0	18
45—49	A	7	4	10	12	6	8	1	2	4	1	55
	B	4	0	1	7	3	3	0	1	3	1	23
50—54	A	13	4	12	14	9	4	2	4	6	3	71
	B	2	0	4	5	4	3	1	2	4	3	28
55—59	A	21	7	13	12	8	10	3	2	3	3	82
	B	4	1	1	5	2	6	0	0	2	1	22
60—64	A	20	4	16	7	9	6	1	2	5	7	77
	B	3	0	1	3	3	1	1	0	3	3	18
	A	76	26	59	56	46	36	9	14	23	15	[illegible]
	B	17	2	7	25	22	19	3	6	17	8	126

Table 13 A. *The percentages returning to work in the different occupational categorie of the material collected from Oulu and Turku. To facilitat comparison the occupational distributions are presented as percentages*

Occupation	Return to work (%) Oulu	Turku	Occupational distribution (%) Oulu	Turku
Unskilled labourers	22.4	11 1	21 1	15.6
Small farmers, lumberjacks	7.7	0	7.2	0.9
Farmers	11 9	10.0	16.4	8.7
Skilled employees in commerce and traffic	44.6	55.3	15.5	13.2
Skilled industrial workers	47.8	36.9	12.8	22.6
Supervisors, foremen	52.8	42.9	10.0	9.7
Small-scal businessman	33.3	45.6	2.5	8.0
Civil servants	42.8	69 1	3 9	7 4
Managerial, academic degree	73 9	39.5	6.4	7.5
Housew es	53 4	44 7	3.2	6.4
Number of cases			360	583

At the other extreme, in the managerial group the *percentage returning to work* was almost twice as high in Oulu as in Turku (73 9 % and 39.5 %, respectively) In the group of minor civil servants there is a similar margin in favour of Turku, the figures being 42.8 % and 69 1 % The differences between the Oulu and Turku figures in the other occupational groups are around 10 %. The average percen tage *return to work in these categories* is about 45 The Sickness Insurance offices in Turku d d not know of any cases among those studied in which it would have been possible for the patient to change into a lighter occu pation on his return to work. No data were obtained from s ckness-benefit funds for such data would not have allowed statistical computations in the present context

There are many factors which affect the resumption of work Place of residence is closely associated with type of occupation (Table 13 A). The material obtained from Oulu clearly more rural as regards occupation, whereas that collected from Turku is predominantly industrial. The rural occupations are restricted in number and changes to other types of work are practically impossible. Re-training is also difficult to arrange for a number of reasons, the most obvious being the low standard of basic education, the long distances involved and the difficult economic situation The situation grows worse as the distance from the population centre in creases. These factors alone greatly restrict the opportunities for patients recovering from myocardial infarction, or other diseases which greatly affect physical performance, to become fully active in their work again It is no exaggeration to say that unless one is able to resume work, *the only other possibility* is to retire on an invalid pension. Many of the small farmers, lumberjacks and farmers in northern Finland have large families and have substantial debts to repay which makes it difficult for them to seek jobs elsewhere, even they might otherwise be able to do so.

The role of the doctor is also highly important. *Doctors vary in their opinions as to* when the working capacity of a patients is so much affected that there is nothing for it but to sign an application for a pension. One may be strict in his interpretation and prescribe only short periods of sick leave, ignoring the patient s occupation and the circumstances under which he would have to resume work. Another may concentrate on the *prevailing* circumstances and social background, thereby overestimating the degree and duration of incapacitation. This is a largely subjective process, which may in one the same case, result in any decision between a few months sick leave and a permanent pension. Apart from this, there is the problem of applying medical knowledge, skill and experience to the treatment of patients recovering from in farction. One knows from experience that in the treatment of some diseases several differ

etho lead to the same result. It

should be noted furthermore, that most doctors have obtained their knowledge of the interaction between the disease and the patient's socio-economic background through the practical procedure of trial and error Only recently has any formal attention been paid to such matters. The training of doctors has mainly concentrated on the medical aspect, ignoring other problems.

Again, the fate of the patient is often influenced by the official who handles the documents concerning his case, one of which is the certificate from the attending physician. This is one factor though not always the decisive one, responsible for the benefits which may be granted to the patient. The bases upon which the benefits of sickness insurance and state pensions are granted in Finland are not identical at present. A person may receive the full sickness insurance allowance for the maximum period of 300 days, and yet not receive any national pension after that, in which case he has to wait for two years before he is again entitled to this allowance. This is due to the different definitions of incapacity contained in the Sickness Insurance Act and the National Pensions Act. This situation may arise unless a complaint is lodged which yield a favourable verdict, or unless there is a substantial deterioration in the patient's health. The final decisions, which are made by the officials of the National Pensions Institute, may be completely erroneous judgements guided more by strict observance of the rules than by common sense. View on myocardial infarction, however have tended to be reconciled at both the upper and the lower levels of the hierarchial social security system during the last few years.

The present study also revealed the remarkable fact that there are hardly any opportunities for a change of occupation in either Oulu or Turku. In this respect the results differ sharply from most of the those reported from other countries. In Sweden, for example, the proportion of those moving to a less demanding occupation has been reported to be 17 %, 27 % or 32 % (Biörck & Trulson 1957 Malmcrona et al. 1962, Biörck & Wedelin 1964) Studies carried out in Finland before the institution of the health insurance scheme in 1964 reported that survivors of myocardial infarction changed to a lighter occupation in 37 % (Rasanen 1951) and 17 % (Sipilä 1966) of the cases.

5 *Effect of income level on resumption of work*

The Sickness Insurance Act came into effect in Finland in 1964 A daily allowance is payable to any insured person aged 16 to 64 who on account of incapacity caused by illness is unable to perform his customary work or any work closely comparable thereto if the insured person has during the three months prior to the onset of his incapacity for work been self-employed or employed by another person. (Statistical Yearbook of the National Pensions Institute of Finland 1968)

This daily allowance was paid at a rate of 0 15 % of annual earned income as assessed for the last completed tax year provided that this income fell between 2750—15000 Fmk per annum in 1964—1966 or 3465—19 500 Fmk per annum in 1966—1968 When this annual income was less than 3465 Fmk, the daily allowance amounted to 4 Fmk in 1964—1966, and 5.20 Fmk in 1966—1968 Any income in excess of 19 500 Fmk per annum was disregarded in the calculation of the daily allowance. The head of a family was further entitled to a provider's supplement, 15 % of the daily allowance for a spouse and 10 % for each dependent child under 16, provided that the total supplement did not exceed 50 % of the daily allowance.

Table 14 shows the distribution of those capable of work at the onset of infarction and those capable of returning to work according to whether they received the basic or in

Table 13 A. *The percentages returning to work in the different occupational categories of the materials collected from Oulu and Turku. To facilitate comparison, the occupational distributions are presented as percentages.*

Occupation	Return to work (%)		Occupational distribution (%)	
	Oulu	Turku	Oulu	Turku
Unskilled labourers	22.4	11 1	21 1	15.6
Small farmers, lumberjacks	7.7	0	7.2	0.9
Farmers	11 9	10.0	16.4	8.7
Skilled employees in commerce and traffic	44.6	33.3	15.5	13.2
Skilled industrial workers	47.8	36.9	12.8	22.6
Supervisors, foremen	52.8	42.9	10.0	9.7
Small-scale businessman	33.3	45.6	2.5	8.0
Civil servants	42.8	69 1	3 9	7 4
Managerial, academic degree	73 9	39.5	6.4	7.5
Housewives	53 4	44.7	4.2	6.4
Number of cases			360	583

At the other extreme, in the managerial group the percentage returning to work was almost twice as high in Oulu as in Turku (73 9 % and 39 5 %, respectively) In the group of minor civil servants there is a similar margin in favour of Turku, the figures being 42 8 % and 69 1 %. The differences between the Oulu and Turku figures in the other occupational groups are around 10 %. The average percentage return to work in these categories is about 45 %. The Sickness Insurance offices in Turku did not know of any cases among those studied in which it would have been possible for the patient to change into a lighter occupation on his return to work. No data were obtained from sickness benefit funds, for such data would not have allowed statistical computations in the present context.

There are many factors which affect the resumption of work. Place of residence is closely associated with type of occupation (Table 13 A). The material obtained from Oulu is clearly more rural as regards occupation, whereas that collected from Turku is predominantly industrial. The rural occupations are restricted in number and changes to other types of work are practically impossible. Re-training is also difficult to arrange for a number of reasons, the most obvious being the low standard of basic education, the long distances involved and the difficult economic situation. The situation grows worse as the distance from the population centre increases. These factors alone greatly restrict the opportunities for patients recovering from myocardial infarction, or other diseases which greatly affect physical performance, to become fully active in their work again. It is no exaggeration to say that unless one is able to resume work, the only other possibility is to retire on an invalid pension Many of the small farmers, lumberjacks and farmers in northern Finland have large families and have substantial debts to repay which makes it difficult for them to seek jobs elsewhere, even they might otherwise be able to do so

The role of the doctor is also highly important. Doctors vary in their opinions as to when the working capacity of a patients is so much affected that there is nothing for it but to sign an application for a pension. One may be strict in his interpretation and prescribe only short periods of sick leave, ignoring the patient's occupation and the circumstances under which he would have to resume work. Another may concentrate on the prevailing circumstances and social background, thereby overestimating the degree and duration of incapacitation This is a largely subjective process, which may in one the same case, result in any decision between a few months' sick leave and a permanent pension. Apart from this, there is the problem of applying medical knowledge, skill and experience to the treatment of patients recovering from infarction. One knows from experience that in the treatment of some diseases several different methods may lead to the same result. It

Table 17 *Occupation and daily allowance in relation to return to work. A = those capable of work at the onset of infarction. B = those returning to work.*

Occupation		Daily allowance in Fmk 4.00–5.20	5.21–8.00	8.01–11.00	11.01–15.00	15.01–20.00	20.01–25.00	25.01–30.00	over 30.00	Not know	Total
Unskilled labourers	A	19	21	12	11	8	4	0	0	1	76
	B	2	5	3	5	2	0	0	0	0	17
Small farmers, lumberjacks	A	8	12	1	4	1	0	0	0	0	26
	B	0	1	0	1	0	0	0	0	0	2
Farmers	A	14	27	10	2	4	2	0	0	0	59
	B	2	1	3	0	0	1	0	0	0	7
Skilled employees in commerce and communications	A	7	7	6	9	21	1	2	2	1	56
	B	0	3	4	5	9	1	2	1	0	25
Skilled industrial workers	A	3	4	2	5	12	16	3	0	1	46
	B	0	2	1	2	6	8	3	0	0	22
Supervisors, foremen	A	2	0	1	5	9	17	1	1	0	36
	B	1	0	1	4	3	9	1	0	0	19
Small-scale businessmen	A	1	0	1	2	1	1	1	1	1	9
	B	0	0	1	0	0	0	1	1	0	3
Civil servants	A	0	0	0	2	5	1	3	3	0	14
	B				0	2	1	2	1	0	6
Managerial position, academic degree	A	0	0	0	0	1	3	8	11	0	23
	B					1	2	5	9	0	17
Housewives	A	15	0	0	0	0	0	0	0	0	15
	B	8									8
Total number of cases	A	69	71	33	40	62	45	18	18	4	360
	B	13	12	13	17	23	22	14	12	0	126

which have already been discussed in chapter 4

A comparison of these results with the previous Finnish studies, which report that over 70 % of the survivors returned to work prior to the establishment of sickness insurance (Rasanen 1951 Lanko 1958 Sipilä 1966) reveals some questions requiring elucidation. Since socio-economic security of the sick was less adequate at that time it is probable that economic reasons forced patients recovering from myocardial infarction to return to their old work earlier often against the orders of their doctors, or if the same job was no longer available, to look for some other work. It is only natural, of course, that the views of doctors have also changed in the course of the years. The present Sickness Insurance Act allows money to be paid for the first 300 days of illness, and if the patient continues an invalid even after that, he is then treated under the National Pensions Act. Especially in the case of the poor who have lived at subsistence level all their lives, sickness insurance guarantees a continuous taxfree income up to the above-mentioned maximum for the duration of illness. For many unskilled workers, small farmers and lumberjacks this in practice means that the standard of living they have enjoyed while working continues unchanged, occasionally even improves. The original work usually being heavy and the

working conditions dismal, it is practically impossible to rehabilitate such a person, or even to suggest rehabilitation, particularly since his health has often deteriorated substantially.

On the other hand, in the opposite we may ask whether the law treats a person justly who has possibly achieved a managerial position by hard work, and whose income exceeds the limit of 19 500 Fmk per annum, for he will receive the fixed maximum sum no matter how high his income may be. And since the higher his income is, the higher his taxes while working, he does not receive the daily allowance he deserves on the basis of his work and his contribution in taxes. This problem is a political one and need not be speculated upon here.

These examples illustrate the emerging difficult problem: how far is it possible to improve social security without simultaneously reducing the convalescent's desire to return to active work. A solution may suggested by a comparison of the percentage return to work obtained previously and the values recorded here.

6 Effect of number of infarctions suffered and occupation on resumption of work

Table 18 records initial infarctions and their effect on resumption of work in the various occupational groups. As can be seen the percentages of those returning to work are more or less similar to the occupational percentages presented above (Table 12) for all infarctions. There were only a few people capable of work at the onset of the second or third attack, and they are presented separately in Table 19. These small values have no statistical effect on the values presented. None of the women were able to work at the onset of the second or third attack. Thus this *discussion must be restricted to men only.* It can be seen that the patients with heavy occupations very seldom resumed work after a recent infarction, let alone after the first or second recidive. The group of less physically demanding occupations, i.e. managerial staff, showed a percentage slightly higher than that for the total material.

Table 18 *Return to work after recent myocardial infarction in the different occupational groups.*

Occupation	Those capable of work at the onset of infarction (A)			Those returning to work (B)			$\frac{B}{A}$ in per cents		
	M	F	Total	M	F	Total	M	F	Total
Unskilled labourer	51	16	67	13	1	14	25.5	6.2	20.9
Small farmers, lumberjacks	21	4	25	2	0	2	9.5	0	8.0
Farmers	46	10	56	5	1	6	10.9	10.0	10.7
Skilled employees, commerce and communication	4	6	46	20	1	21	50.0	16.7	45.6
Skilled and retail workers	36	3	39	17	2	19	47.2	66.7	48.7
Supervisors, foremen	33	—	33	15	—	15	45.5	—	45.5
Small-scale businessmen	5	3	8	2	1	3	40.0	33.3	37.5
Civil servants	1	1	11	6	0	6	60.0	0	54.6
Managerial position, academic degree	2	1	21	16	1	17	80.0	100.0	81.0
Housewives	—	13	13	—	8	8	—	61.5	61.5
Total	[illegible]42	57	319	96	15	111	36.6	26.3	34.8

Table 19 *Return to work after the first and second recidives in the different occupational groups. Only men.*

Occupation	Those capable of work at the onset of the		Those returning to work after the	
	first recidive	second recidiv	first recidi	second recidive
Unskilled labourers	9	—	3	—
Small farmers, lumberjacks	1	—	0	—
Farmers	2	—	1	—
Skilled employees in commerce and communications	5	3	3	1
Skilled industrial workers	5	—	3	—
Supervisors, foremen	4	1	4	0
Small-scale businessmen	1	—	0	—
Civil servants	3	—	0	—
Managerial, position, academic degree	2	—	0	—
Total no. of cases	32	4	14	1

7 *Duration of sick-leave*

Table 20 shows cumulatively the percentages returning to work after recent myocardial infarction The percentages are calculated from the numbers of those able to work at the onset of the infarction.

None of the patients who received daily allowance for the maximum of 300 days (10 months) returned to work afterwards. In other words, unless a patient returned to work within 10 months, he did not do so at all. This applies to the occupation held by the patient at the onset of the infarction.

The total material contained 32 men who were able to work at the onset of the first recidive. Of these 14 (43.7 %) returned to work 4 within 3 months after the attack, another 4 within the next 3 months, and 6 within the remaining 4 months making up the appointed period of 10 months. The whole material contained only one man who returned to work after the second recidive attack, and he did so within 3 months.

Table 21 shows cumulatively the duration of sick leave among those returning to work after recent myocardial infarction.

Tables 20 and 21 show that men return to work more frequently and after a shorter period of convalescence following recent myocardial infarction than women do Most of the survivors (about 95 %) resumed work within 6 months.

Comment

Master & Dack (1940) report that 54 % of the 169 patients for whom the time of return to work following myocardial infarction could be determined resumed work within 3 months, 76.5 % did so within 6 months, and 92 % within one year of discharge. Ten patients continued to work throughout the entire attack, the diagnosis being made later on the basis of the history and electrocardiographic changes. These figures are more or less similar to those obtained in the present study Most of the works dealing with this problem seem to establish six months as the time limit by which most of the patients will have returned to work (Iisalo et al. 1958 Knutsen 1960 Malmcrona et al 1961 Sharland 1964 Weinblatt et al 1966 Kellermann et al 1968) In the cases described by Kellermann et al., most of those returning to work did so within one year Räsänen (1951) described a few cases who returned only after two years sick leave.

The greater the number of infarctions suffered, the poorer the chances of resuming work. Master & Dack (1940) have 59 % of their patients returning to work after recent infarction, 38 % after the first recidive, and only 23 % after the second recidive. Weiss (1961) noted that 62 % of male survivors of a second infarct returned to gainful employment. The 120 cases of patients able to work presented by Biörck & Wedelin (1964) contained 10 men who were able to return to their previous work after 2 infarctions, and

Table 22. *Return to work after recent myocardial infarction cumulatively after the different durations of sick-leave.*

Duration of sick-lea	Males		Females		Total	
1— 3 months	64	(24.4 %)	8	(14.0)	72	(22.6 %)
4— 6 months	92	(35.1)	14	(24.6 %)	106	(33.3)
7—10 months	96	(36.7)	15	(26.4 %)	111	(33.8 %)
Number of those capable of work at the onset of recent infarction	262		57		319	

Table. 21 *Cumulative return to work after different periods of sick-leave among those who recovered their working capacity after recent myocardial infarction.*

Duration of sick leave	Males		Females		Total	
1— 3 months	64	(66.7)	8	(53.3 %)	72	(64.9)
4— 6 months	92	(96.0 %)	14	(93.5 %)	106	(95.5 %)
7—10 months	96	(100.0 %)	15	(100.0 %)	111	(100.0 %)

no less than 4 who were able to do so after 4 infarctions. In the present material only one man was able to resume his work after the first recidive, despite the fact that the mean age was here lower than that of the Swedish cases.

In spite of the opportunities for rehabilitation and retraining provided by the sickness insurance system, none of the patients in the present material was in any way helped by this system in finding a new lighter occupation. The situation prevailing among patients with myocardial infarction resuming work is now widely different from that revealed in earlier Finnish studies, which show higher percentages for return to work, and similarly a large number of patients taking less demanding jobs (Rasänen 1951 Iisalo et al. 1958 Sipila 1966)

VII DISCUSSION

The present study concerns resumption of work after myocardial infarction amongst hospital patients. The results, therefore, are not strictly accurate, since victims of myocardial infarction who died at their work, at home or on their way to hospital are excluded. If there had been a coronary register available, the situation would have been somewhat easier to evaluate. It should also be pointed out that some coronary patients die from arrhytmia without any morfological changes observable at obduction (Biörck 1960, Hinkle 1968, Edwards 1969 1971) In practice, therefore, a set of hospital patients is the only reliable material. As regards the present work, it should also be emphasized that most of the cases of acute myocardial infarction, and especially those amongst the working population, were treated in hospital.

Certain aspects of these results have been compared with corresponding data obtained from the University Central Hospital in Turku concerning the same period of time. The Oulu District Hospital and the University Central Hospital in Turku hold comparable positions in their areas: they receive all the local cases of acute myocardial infarction Turku is fairly representative of southern Finland, which is more prosperous and more widely industrialized and has a better employment situation than northern Finland.

The present study has been restricted to deal with resumption of work after myocardial infarction without any consideration of the medical aspects of myocardial infarction such as the nature, location, or severity of the muscular lesion, or the nature of cardiac function following infarction. It is obvious that infarctions resulting in serious congestive heart failure much more frequently prevent the patients from returning to work than do infarctions that pass without complications. Yet resumption of work after myocardial infarction is not only a problem of somatic medicine. Many other factors have their part to play as well. There are, first of all, psychological factors, such as the attitude of the attending physician towards the patient's dilemma, and the patient's own problems, which are exceptionally pressing after such a dramatic experience as myocardial infarction. Further significant factors include the attitude of the patient's family the role played by the employer and the chances of society helping the patient to return to work. These problems are discussed by Lewis (1968) who investigated return to work after congestive heart failure, and Goble et al. (1963) Lovell (1964) Carroll (1968) Rantalaiho (1968) Hinkle (1969) and Miller & Brewer (1969) Brown et al. (1969) Nagle et al. (1971), who concentrate on myocardial infarction. If the question is treated as one large scale problem, it can be seen that a detailed medical classification of myocardial infarctions is of no significance the concept of myocardial infarction as one disease is an adequate basis for the discussion of return to work

The resumption of work after myocardial infarction has previously been studied from the point of view of age, sex, marital status, place of residence, occupation, income level, number of infarctions suffered and duration

of sick leave. These factors are associated in many ways. When the rehabilitation of a patient recovering from myocardial infarction begins, it is useful to study the factors affecting return to work in quite a different manner. There are four possible approaches.

1. *Medical factors* impose their own restrictions, as already mentioned, but they are not the only ones. Other possible diseases must be taken into account, and the situation must thereafter be viewed as a whole.

The age of the patient is very important. The older the patient, the lower generally speaking, the chances of resuming work. The present results show a clear decline after 50—54 years, around which point the percentage return to work drops from 40 % to little more than 20 %. Some Australian studies show this critical line to be somewhat lower: patients over 45 years of age made a poorer recovery (Morgan Jones, 1957) or had difficulties in resettlement (Miller 1967).

Women in addition to suffering myocardial infarctions at an older average age than men do also return to work less frequently (Levine & Rosenbaum 1941, Biorck & Trulson 1957, Biorck 1964, Lund—Johansen 1965, Kellermann et al. 1968). Biorck (1959) therefore suggests that it might be advisable to give women instruction in everyday household work while they are still in hospital recovering from myocardial infarction. This is hardly feasible in practice, but the problem remains urgent, as is shown by the present study: 53 % of the housewives in the Oulu material and only about 45 % in the Turku material regained their full working capacity. This is all the more significant, as most of the women labelled as housewives live in towns, where modern household facilities are available for almost everyone. Without going into the psychological problems, it can be acknowledged that doctors generally take a much more reserved view towards the need of sick leave and demand for rehabilitation of female patients, to say nothing about their attitude towards women applying for pensions.

2. *The role of the doctor* is not the only crucial factor determining return to work, although it is quite a central one. The doctor has the constant problem of what information should be given to a patient recovering from myocardial infarction, and how that information should be imparted. The difference between benefit and harm in the communication of information is often very slight, and even an experienced doctor may be mistaken in his judgement, thereby causing a favourable piece of information to be interpreted wrongly and consequently bringing about more harm than good (Goble et al. 1963, Lovell 1964). The better educated the patient is, the easier it is for him to obtain the necessary information about his illness even from limited data. The language of communication between such a patient and the doctor is more intimate, and the doctor need not adopt the patient's vocabulary. Nevertheless, it should always be remembered that the doctor may overestimate the power of comprehension of such a patient, and therefore give him an erroneous view of his illness. Croog & Levine (1969) noted that members of the higher social classes are better informed about their illness, partly for the reasons enumerated above.

In his study published as early as 1939 White pointed out that the patient should not be needlessly frightened by being told about the possible complications of myocardial infarction or by being given a pessimistic view about his future. According to the editorial of the British Medical Journal (BMJ 1964) "there is still far too much doctor induced neurotic ill-health among those who have suffered from myocardial infarction".

But neither is excessive optimism a particularly good thing. It may happen that the patient, after returning home, is unable to per

form at the level expected or that his family is unable to supply the services promised (Carroll 1968) An attitude of optimism however is essential (BMJ 1964 Carrol 1968 Miller & Brewer 1969) but how to establish this is another question. The doctor should be able to view the situation from the position of the patient, which would also help him to find the correct language of communication. He should further arrange enough time for the interview a matter which, according to Biörck (1959) is frequently overlooked by doctors. The instructions for treatment, whether given to the patient or to his family should be simplified, for the greater the number of directions given, the less likely they are to be carried out. This also applies to the number of medications prescribed (Carrol 1968)

The attitude of doctors towards myocardial infarction varies greatly Exceedingly long treatment tends to reduce the patient's motivation to return to work, as he begins to adapt to the role of an invalid. On the other hand, exceedingly short treatment aiming at an early return to work may have precisely the same effect. The initial duration of sick leave is, in the first instance, determined by the experience and personal opinion of the doctor and these are subject to great variation. The present study showed that some doctors prescribed sick leave in spells of one or two months, adding to the duration after each such spell if necessary while some others immediately considered a convalescent period of six months to be necessary in similar cases. The material collected from Turku seems to show the same pattern. It has already been mentioned that there is often disagreement between the sickness insurance doctor and the doctor working for the National Pensions Institute concerning one and the same case. In addition to the Sickness Insurance Act and the National Pensions Act being different in their definitions on incapacity divergent interpretations of one case even occur on the medical side. No investigations concerning this vital aspect have so far been available.

The question of how strongly the doctor should urge his patient to return to work is answered differently in different cultures. Biörck (1964) points out that the average life-time remaining after myocardial infarction is ten years, which is also the average life-time remaining for people retiring from work in the Western countries. Biörck therefore poses the following question would it not be possible to grant the survivors of myocardial infarction a freedom of retirement similar to that of pensioners, particularly since unemployment is a constant threat and young people should be given their chance, instead of wasting money and energy trying to rehabilitate patients to resume work?

One additional factor deserving attention is that neither the doctors working for the health insurance system nor those employed by the National Pensions Institute can exercise any control over the work of the attending physician i.e. they cannot examine the patient themselves. The only way to control the work of a particular doctor is to refer the patient to a third doctor But as this procedure is expensive and only rarely applicable in practice, the only procedures open to the inspecting doctor are either to accept the data as true and grant the required benefit or to reject the application. Under these circumstances it may happen that a doctor's certificate with inadequate data or formal imperfections may cause the application to be rejected without any real justification. This only serves to emphasize further the subjective aspect of the doctor's role.

In the light of the present results Biörck's idea does not seem so far fetched. Broadly speaking, if a manual worker in northern Finland suffers myocardial infarction, he cannot recover sufficiently to return to work, because opportunities for re-education or for transferring to a new job are nonexistent.

According to BMJ (1964) it is in general best for the patient to return to his usual job unless it involves heavy manual work.

It has already been noted above that age has a great effect on return to work, irrespective of occupational group Thus patients who are near retiring age may with good reason decide that a cardiac infarct is an adequate reason to retire early and they should be helped to do so if this is their wish (BMJ 1964) The journal further argues that there is little point in urging a man to retire if he wants to go on working — in fact this is more likely to do harm than good. No such eagerness to work was noted in the present material

3 *The role of the patient* has been touched upon earlier Certain *personality defects* such as psychopathies, are in themselves sufficient to frustrate efforts to encourage a return to work (Galea 1960 *Miller & Brewer 1969*) Biörck (1959), in his discussion of the social and psychological problems encountered among patients with chronic heart diseases, maintains that many of the patients prefer to continue their own work, even when it is physically demanding, rather than change to another occupation which they are not familiar with, and which may therefore lead to conflicts with fellow workers and difficulties in adaptation. Similar difficulties have been observed by Miller & Brewer (1969) particularly among patients who have obtained skills or experience in heavy manual work, in which status and economic security have often depended on the patient s physical strength With the diminution of this asset, these people are initially unprepared to accept the necessity of reducing their income. However many patients after a prolonged illness tend to revert to a dependent personality relying heavily on the social services available to them, and not daring to leave the safety of this sustenance This is in complete agreement with the present results.

Motivation to resume work may be suppressed in two different ways. Firstly there are people who deny their illness and refuse to assume the role of an invalid which relieves the patient of all his responsibilities and obligations. The patient is expected to allow himself to be cared for by others, but he is also expected to relinquish this role as soon as possible (Carrol 1968)

Secondly there are patients who accept the role of an invalid. They are often people who want to escape from the prevailing circumstances, which present difficulties for them. Thus a heart attack may turn out to be a socially acceptable reason for retiring from business or withdrawing from the rough and tumble of lige. Such passive sickness-prone patients accept this role eagerly and are reluctant to relinquish it (Carrol 1968 Rantalaiho 1968)

Lack of motivation to resume work may be due to various reasons. Several authors have suggested that fear of another attack or of sudden death may greatly impede the recovery of survivors of myocardial infarction (Biörck & Trulson 1957 Goble et al. 1963 Lovell 1964 Lund—Johansen 1965 Wynn 1967 Carrol 1968 Miller & Brewer 1969 Lancet 1971) The kind of misunderstanding between the doctor and the patient discussed above may lead to a lack of motivation. The patient s family may similarly live in fear of a fresh attack and prevent the patient s rehabilitation by their over anxious protection and care (Klein et al. 1965 Lewis 1968) The family may change their attitude or lose enthusiasm when they find out what is required to fulfil the patient s needs at home (Carrol 1968)

Neighbours, employers and workmates all have their own ideas about and attitudes towards those who suffer from heart disease The patient, having been conditioned throughout his life to these concepts, accepts all shorts of fallacies because they agree with his own ideas. The situation grows worse if

there is no authoritative medical advice available, and the patient may believe the most remarkable ideas about his condition (Goble et al. 1963) Such misconseptions are often firmly roated in the patient's mind, and if they have been accepted as facts for a long time, it becomes increasingly difficult to eradicate them.

Among the lower social classes the lack of motivation may further be influenced by the social security benefits provided. These may occasionally exceed the wages earned by working (Miller & Brewer 1969) This can be seen in the present material, particularly *among the unskilled workers* and those receiving a provider's supplement. Such a situation may impede the development of the social security system. In the case of myocardial infarction the situation is even more difficult. the condition itself may be insignificant medically but publicity has given myocardial infarction an image which allows withdrawal from work for the slightest medical cause. We are therefore justified in asking whether »excessive» social security diminishes the desire to return to work. On the other hand, adequate social security is naturally an essential prerequisite for the activity of an individual (Jyrkilä 1965 Carrol 1968 Rantalaiho 1968 Brown et al. 1969).

4 *The role of society* has been partly dealt with in the previous section. A patient recovering from a serious illness like myocardial infarction is nowadays very seldom able to transfer to a less demanding occupation. Change of job is generally easier in the higher social classes, whose members are better educated and therefore more adaptable. This »social exchange» is more common in the higher social classes. In order to be successful, this social exchange requires that several alternative occupations should be available, which in turn would also improve the opportunities for the lower social classes to return to work. Since, however no alternatives are available, the less educated and unskilled workers are the first to be excluded from active work. The situation is further aggravated by unemployment, which may strike down not only convalescents but also completely healthy people. This brings us to the pressing problem of the role played by the doctors in the arrangement of a patient's social security Nearly every practicing physician is familiar with the situation of a patient who is medically fit to work, but who, in the absence of a job, is forced to resort to society for economic support. Employment officers and social workers generally send the person *to be examined by a doctor* hoping that some grounds might be found for a daily allowance or pension If the doctor is unable to find anything that would justify the awarding of such benefits, the patient is left at the mercy of the social security board It is also possible that the doctor may find some minor illness characteristic of survivors of myocardial infarction If this is the case, the patient is not even accepted onto the files of the unemployed applying for work. The resulting situation is the same social insecurity and conflicts between the doctor the patient and society

Against this background it seems that there is not much a doctor can do but sign the application for a permanent pension if the patient recovering from myocardial infarction is not able to return to work. Even this is frequently left for the patient do decide, which means that medical grounds play a very minor role.

The economic structure of southern Finland differs from that of northern Finland: the labour force in the south is better trained and the economic structure is more industrial in character This is also shown by a comparison of myocardial infarction cases in Oulu and Turku. It is interesting to see that the people in the managerial and supervisory groups and other skilled industrial workers return to work less frequently in the south than in the north. The age structure being the same in the two materials, this finding would fit in

with the observation that there is a larger trained labour force available in the south, and it is therefore easier to replace sick employees, whose pensions from firms and industrial establisments are also fairly good. On the other hand some of the unskilled workers receive such poor benefits from health insurance that they prefer to return to work. The number of these is twice as great in the northern material as in the material collected from Turku. There is, however another factor as well the clients of the social security boards are proportionally more numerous in Oulu than in Turku, so that support must be more difficult to obtain in Oulu, and people are therefore forced to return to work.

Rehabilitation in general and particularly the measures taken after myocardial infarction, is still open to many questions. Different social groups hold different views concerning rehabilitation and the matter can thus be treated in political terms. The medical benefits of rehabilitation following myocardial infarction are largely unknown. This is particularly true in the evaluation of physical performance. It should also be remembered that rehabilitation includes all the various forms of re-training

The Sickness Insurance Act and the National Pensions Act include the possibility of rehabilitating and re-training a person for a new occupation. None of the patients in the present study have yet obtained new jobs through this system Moreover the occupations for which training is available are quite restricted in character and medical grounds alone tend to eliminate a large number of patients with myocardial infarction. It has also been noted in practice that retraining is difficult with people past middle ages. The people chosen for re-training should be fairly young, preferably less than 40 years old they should have some kind of basic education, e.g. complete 8 year primary education, they should have no family and be able to move away from the locality where they are living. These criteria in practice disqualify all the myocardial infarction patients living in northern Finland. The proportion of patients with families in the present material was nearly 80 %.

It has been reported that unskilled workers living in the country make the poorest recovery (Purola et al 1967 Purola 1967) This may be true in some cases, but it should also be realized that although sickness insurance enables people to travel practically free of charge and reimburses most of the expenses of examination and treatment, not everyone takes advantage of this. This question has become a major topic of political propaganda, in which the blame is easily imputed to the medical personnel.

In the past, the better the chances for social exchange, the more frequently it has been possible to annul invalid pensions. Rinne & Sarjamo (1966), who base their statement on the statistics of the National Pensions Institute report that annulments are least frequent in the case of cardiac and vascular diseases (0 19 %) while they are more numerous for other diseases (0 73 %). If a person had been received a pension for a cardiac disease, the pension was usually not discontinued. In the present material all those who received a daily allowance for the fixed maximum period of 10 months subsequently became permanent recipients of a national pension.

VIII SUMMARY

The resumption of work after myocardial infarction and the socio-economic factors influencing it were investigated in a subarctic area in northern Finland.

The investigation covers 868 myocardial infarction cases treated in the Oulu District Hospital during the period 1. 1. 1966—30. 4. 1970. There were 661 recent myocardial infarctions, 154 first recidives, 45 second recidives, 6 third recidives and 2 fourth recidives.

The mean age for the total material was 57.4 years, 55.3 years for the men and 64.6 years for the women. The ages ranged from 29 to 92 years. There were 644 men and 224 women, the male-to-female ratio being 2.9. This ratio was 2.6 in the group of recent infarctions, 3.8 amongst the first recidives and 4.9 amongst the second or higher recidives.

Of the total material, 360 (41.5 %) were able to work at the onset of the infarction, 46 (5.3 %) were on sick leave, 3 (0.3 %) were receiving employment pension while still under 65, 159 (18.3 %) were incapacitated by illness while under 65, and 300 (34.6 %) were receiving old age pensions being over 65 years of age. 46.3 % of the men were capable of work at the onset of the infarction, and 29.7 % were receiving old age pensions. The corresponding figures for women were 27.7 % and 48.7 %.

The discussion of return to work concerns only those patients under 65 years of age.

The effect of place of residence on return to work was very noticeable. 50 % of the urban men and 31.6 % the urban women returned to work. The corresponding figures for the rural cases were 23.4 % and 13 %. These differences are statistically significant.

Skilled workers and members of the higher social classes returned to work the most frequently. The people in the highest socio-economic group, those in managerial positions with academic degrees returned to work highly significantly more often than any of the other nine socio-economic groups. In this group 74 % of those capable of work at the onset of the infarction were able to return to work, 7.7 % of the small farmers returned to work, while 11.9 % of the farmers and 22.4 % of the unskilled workers did so. In the remaining socio-economic groups an average of 45 % returned to work.

Nearly all these returned to their previous occupations. Only 3 patients were able to transfer to a less demanding job. The lack of opportunity to choose a new occupation is due to many social factors, such as the unbalanced economic structure, inadequate occupational training, and unemployment.

Only 35 % of all those capable of work returned after myocardial infarction. The percentage, calculated from the number of those who were initially able to work and survived myocardial infarction, is 41.6 %.

About 35 % returned to work after recent myocardial infarction, the corresponding figures for men and women separately being 36.6 % and 26.3 %. None of the women returned to work after first, second or more recidives. Neither did any of the men in the heavy physical occupations after the first or subsequent recidive.

61 % of those who returned to work did so within 3 months following the attack, most of them being members of the higher socio-economic classes. If a patient had not returned to work within 10 months, he did not do so at all but retired on pension regardless of his age, sex and occupation.

The present investigations showed return to work to be of the same order of magnitude as in data compiled from a hospital in Turku, in southern Finland The percentages returning to work were 35.0 % and 37.2 %. These values are clearly poorer than those previously reported in the literature.

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Unto Vuopala

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